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The Biology and Propagation of 'Zostera marina', Eelgrass, in the Chesapeake Bay, Virginia

Virginia Inst. of Marine Science
Gloucester Point

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THE BIOLOGY AND PROPAGATION OF ZOSTERA MARINA, EELGRASS, IN THE CHESAPEAKE BAY, VIRGINIA*

by

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16. ABSTRACT

Basic biological aspects related to the growth and propagation of eelgrass in the lower Chesapeake Bay were studied in a series of six experiments designed to reveal information on seasonal aspects of standing crops, reproduction, transplanting and spontaneous revegetation in denuded areas, and growth of eelgrass seedlings under laboratory conditions of increased nutrient enrichment.

Data analysis revealed distinct seasonal trends in the growth cycle of eelgrass. Transplantation of eelgrass plugs in the fall insures greater survivability than doing so in other seasons. The primary method of revegetation by *Ruppia* sp. and *Zostera* sp. seems to be by lateral growth from adjacent unimpacted areas. The addition of a balanced formulation of fertilizer stimulates the growth of eelgrass under laboratory conditions.

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We would also like to acknowledge the many people on the Chesapeake Bay Program staff who provided assistance when things seemed to stall, in particular, Bill Cook, our program manager, Bert Brun and Walt Valentine.

PREFACE

The Zostera marina (eelgrass) community is one of the most valuable natural resources in the Chesapeake Bay as well as in other shallow water coastal areas. This community serves multiple functional roles in coastal ecosystems. It contains a very dense macroinvertebrate community, which may be the most diverse community in the Bay region. Z. marina and associated organisms are consumed by migratory waterfowl, such as brant, black ducks, wigeon, and scaups while juvenile fishes and blue crabs find both shelter and food in eelgrass beds. Based on preliminary data, Z. marina beds in the Chesapeake Bay may be one of the most significant nursery areas for the blue crab (Callinectes sapidus). The grass beds trap sediments and absorb wave energy, thereby reducing shoreline erosion. This becomes most evident after the loss of a grass bed from a particular location.

Zostera marina beds are important in biogeochemical cycling of estuaries and the nutrients released by Z. marina leaves may be utilized by epiphytic algae which contributes significantly to the overall primary production of the system. Perhaps one of the most important characteristics of Z. marina is its contribution to the detrital food chain.

Zostera marina has historically been beset with major catastrophes. In the most well documented decline in the 1030's a disease epidemic destroyed most Z. marina beds on the east coast of the United States and elsewhere in the world. In many areas, including the seaside of Eastern Shore, Virginia, Z. marina has still not reestablished. Bay scallops, which depend on Z. marina for attachment of larvae, have largely disappeared from Virginia waters because of its disappearance.

More recently in the early 1970's vast areas of Zostera marina have disappeared in the lower Bay and in particular the main rivers entering the Bay proper, e.g. the York and Rappahannock rivers. Various causes for the decline have been hypothesized e.g. climatic changes, increased runoff, with resultant increased sediment load and thus reduced light intensity, herbicides and increased epiphytic loads. However, there has been no substantial evidence to date linking any specific cause to the decline in recent years.

Less extensive losses result from human disturbances. Dredging and boating activity have been shown to have a negative impact on established beds, creating large, bare areas in the bed.

Because of the large decline of Zostera marina in the Bay, there has been increasing interest among people from both the private and public sector for replanting this species. Some recent studies have shown that Z. marina beds can, under suitable conditions, recover naturally either by vegetative growth from remaining plants, or by growth of seedlings. However, initial recolonization is often slow or fails to occur before changes in the exposed sediment preclude regrowth. Given the important ecological role of *Z. marina*, it is desirable to be able to assist the early stages of the recovery process. It may also be desirable to attempt to reestablish beds on the Eastern Shore where regrowth has not occurred since the 1930 disease epidemic. Success in such an endeavor could allow reestablishment of Bay scallop populations.

Prior to 1978, relatively little was known about the biology and ecology of *Zostera marina* in the Chesapeake Bay. Most of the previous studies involving this species in the Bay area were concerned primarily with the associated faunal communities. Very little information was available on its phenology, the seasonal aspects of standing crop, productivity, nutrient and light requirements, reproductive periodicity, and its distribution and abundance in both the past and present. In addition, with the decline of *Z. marina* beds in many aras of the lower Bay, interest was being generated on the possible use of transplanting *Z. marina* to reestablish denuded areas.

Because of the importance of *Zostera marina* and other species of submerged aquatic vegetation in the Bay and the decline of these species in the 1970's, the EPA's Chesapeake Bay Program identified submerged aquatic vegetation as a high priority area of research. Because one of the major aims of the program was to translate the information generated by the researchers into an effective management program, we felt that much of the basic life history information of *Z. marina* would be necessary for any effective management scheme.

This project was conceived and carried out in terms of trying to elucidate some of the basic biological aspects of the growth of *Zostera marina* in the lower Chesapeake Bay. Combined with other research programs in Virginia on the functional ecology of *Z. marina* and the distribution and abundance of submerged aquatic vegetation, the ultimate results of all the reseach would be a more complete understanding of the biology and ecology of *Z. marina* in the Bay.

Because of the number of subprojects that were carried out during the course of this program, we have presented each subproject as a unit in itself, with their own introduction, methods, results, discussion and literature cited. We felt that this approach would make for easier presentation of the large amount of data generated here as well as to make it easier for later publication in the scientific literature. Each chapter will be redrafted prior to submission to a scientific journal in order to update the literature cited section. Several chapters have already been submitted to peer-review journals and two have been accepted. Appropriate citations are given in these Chapters.

The data from the "Biology and Propagation of *Zostera marina*" program is thus presented in the following sections:

1. Seasonal aspects in standing crop.

2. Anthesis and seed production.
3. Seed germination and seedling growth.
4. The effects of transplanting *Zostera marina* to recently denuded areas.
5. Regrowth of submerged vegetation into a recently denuded area caused by boat disturbance.
6. Growth of *Zostera marina* seedlings under laboratory conditions of increased nutrient enrichment.

CHAPTER 1

SEASONAL ASPECTS IN THE STANDING CROP OF *ZOSTERA MARINA* IN THE LOWER CHESAPEAKE BAY

by

Robert J. Orth
and
Kenneth A. Moore

ABSTRACT

Seasonal aspects of the standing crop of Zostera marina leaves and roots and rhizomes, leaf length and shoot density were measured at five sites in three locations in the lower Chesapeake Bay: one site at Browns Bay in the Mobjack Bay and two sites each at the Guinea Marshes located at the mouth of the York River and Vaucluse Shores located on the Eastern Shore. Sampling at most sites occurred from June 1978 to July 1980. Shoot density, mean leaf length and total standing crop of Ruppia maritima were also obtained at one of the Vaucluse Shores sites and at the Browns Bay site.

The standing crop of Zostera marina vegetative shoots increased in the spring of each year and was hightest in the June-July period at all sites. Minimal values for standing crop occurred during the fall-winter period in both years. Differences in standing crop were found between years for similar time periods at each site. Root-rhizome standing crop followed similar trends as the shoot standing crop. Reproductive shoots made up less than 25% of the total number of shoots during the spring period when they were present. Lowest density of shoots occurred in the late summer and early fall while highest density occurred in the spring and early summer months although there was some variation at several of the sites.

Growth of Zostera marina appeared to occur primarily from late September to early July as temperatures ranged from 0°C to 25°C. Almost no growth occurred in late July, August or early September when no new shoots were observed and when water temperatures exceeded 25°C. Comparison of these data with data collected from sites along the East Coast of the U.S. indicated similarities in the growth cycles at all sites except that maximum standing crop measurements were attained earlier in more southern areas and later in more northern locations. Temperature appeared to control growth although results from recent studies indicate that irradiance is also critical in determining timing of leaf growth.

Rupia maritima also exhibited distinct trends in seasonal standing stock measurements. Growth patterns were similar to Zostera marina except that maximum values occurred later in the summer while minimal values were obtained in March. The reproductive phase also occurred in the summer after Z. marina had been completed.

INTRODUCTION

Zostera marina (eelgrass) is the most abundant species of seagrass found along the entire east coast of the United States. Until recent years, Z. marina in the Chesapeake Bay was very abundant from the Hampton Roads area in Virginia to Eastern Bay in Maryland. Despite recent declines baywide in this distribution and abundance (Orth et al., 1979; Anderson and Macomber, 1980), Z. marina is still abundant in a few areas.

In the Chesapeake Bay and elsewhere, Zostera marina is important as a nursery and habitat for vertebrate and invertebrate species (some of which are of commercial value, e.g. the blue crab, Callinectes sapidus), it can act as a nutrient pump, a shoreline erosion control mechanism and a source of detritus (Wood et al., 1969; Phillips, 1974; Thayer et al., 1975).

Zostera marina is a marine angiosperm and is one of approximately 55 species of seagrasses found in the world today (den Hartog, 1970). The life cycle of Z. marina is quite similar to plants on land, reproducing both vegetatively from rhizome stock and sexually fom seeds. Setchell (1929) was the first to describe the phenology of Z. marina in North America and related the growth and reproduction to temperature. Recently, there have been a number of studies conducted on the biology of Z. marina worldwide that add significant information to its life history (McRoy, 1966, 1970; Phillips, 1972; Jacobs, 1974; Aioi, 1980; Mukai et al., 1979).

Within the Chesapeake Bay, studies on Zostera marina have been primarily limited to the associated faunal communities (Marsh, 1973, 1976; Orth, 1973, 1977) and very little data are available on its phenology. Data from Marsh's (1970) study represented the only seasonal study on changes in the standing crop of Z. marina available for the Bay region.

Because of the paucity of information in the Bay for Zostera marina, the objective of this study was to describe the seasonal changes that occur in the standing crop of both vegetative and reproductive Z. marina from a variety of locations in the lower Bay. The information generated here complements the data collected by the functional ecology program (EPA Grant No. 805974) where other data were being taken simultaneously to this work.

STUDY SITES

Three areas in the lower Chesapeake Bay were chosen as sites for delineating seasonal changes in standing crop of Zostera marina as well as describing its reproductive biology: an area near the mouth of Browns Bay in the Mobjack Bay; adjacent to the Gunea Marshes at the mouth of the York

River; Vaucluse Shore at the mouth of Hungars Creek on the Eastern Shore of the Chesapeake Bay (Fig. 1).

Browns Bay represents a mixed assemblage of *Zostera marina* and widgeon grass (*Ruppia maritima*). The vegetation is found in a band adjacent to the shoreline in a bed approximately 400 meters wide. About 407,500 m^2 of bottom are covered by vegetation in the immediate vicinity of this study site. Biomass data for this site were collected beginning in October 1979.

Guinea Marsh, where two stations are located (one nearshore and one offshore), represents an assemblage in which *Zostera marina* is the predominant species. *Ruppia maritima* is found only in scattered amounts in the shallowest nearshore areas. The area surrounding the Guinea Marshes is a vast shoal area where we have estimated 3,087,600 m^2 of bottom to be covered by vegetation. Biomass collections were initiated in the offshore site in June 1978 while the nearshore site was established in April 1979.

The vegetation at Vaucluse Shores exists between the shoreline and an offshore sandbar located 700 meters from shore at its maximum width (total area is approximately 2,105,000 m^2). The persistence of this grass bed is largely due to the presence of this offshore bar. *Ruppia martima* predominates the inshore shallow areas and *Zostera marina* predominates the deeper sections of the bed (>1m). Both species are found at intermediate depths. Initially, samples were collected in the *Z. marina* areas only. Subsequently, two additional stations were established in May 1979, one in *R. maritima* and one in the mixed zone. These two additional stations were chosen to complement work being done in the EPA-SAV Functional Ecology Program.

METHODS AND MATERIALS

Monthly samples for biomass measurements were initially taken from a homogeneous section of the *Zostera marina* bed. A 0.1 m^2 ring was placed on the bottom and all the vegetation including the roots and rhizomes were removed by hand to a depth of approximately 10 cm. Four samples were normally collected monthly at each sampling location. In June 1979, a 0.033 m^2 core was adopted for sampling the vegetation. A comparison of the data collected using these 2 methods at two different sites revealed little differences for the parameters being measured with the vegetation (Table 1). Subsequently six cores of vegetation were taken at each site. Beginning in January 1980, only three cores were taken at each site.

After removal of the vegetation and sediment from the ring or core all material was placed in a cloth mesh bag and washed free of all sediment. Roots, rhizomes and leaves were then placed in another bag and held in running water until processed within 24 to 48 hours. Processing included: 1) separating the shoots from the roots and rhizomes; 2) counting all shoots and measuring 100 for length and 20 for width; 3) counting reproductive shoots (when present) and recording length; 4) drying roots, rhizomes and leaves for 48 hours at 45°C; 5) placing the material in a dessicator after drying to allow cooling to room temperature; and 6) weighing the material to the

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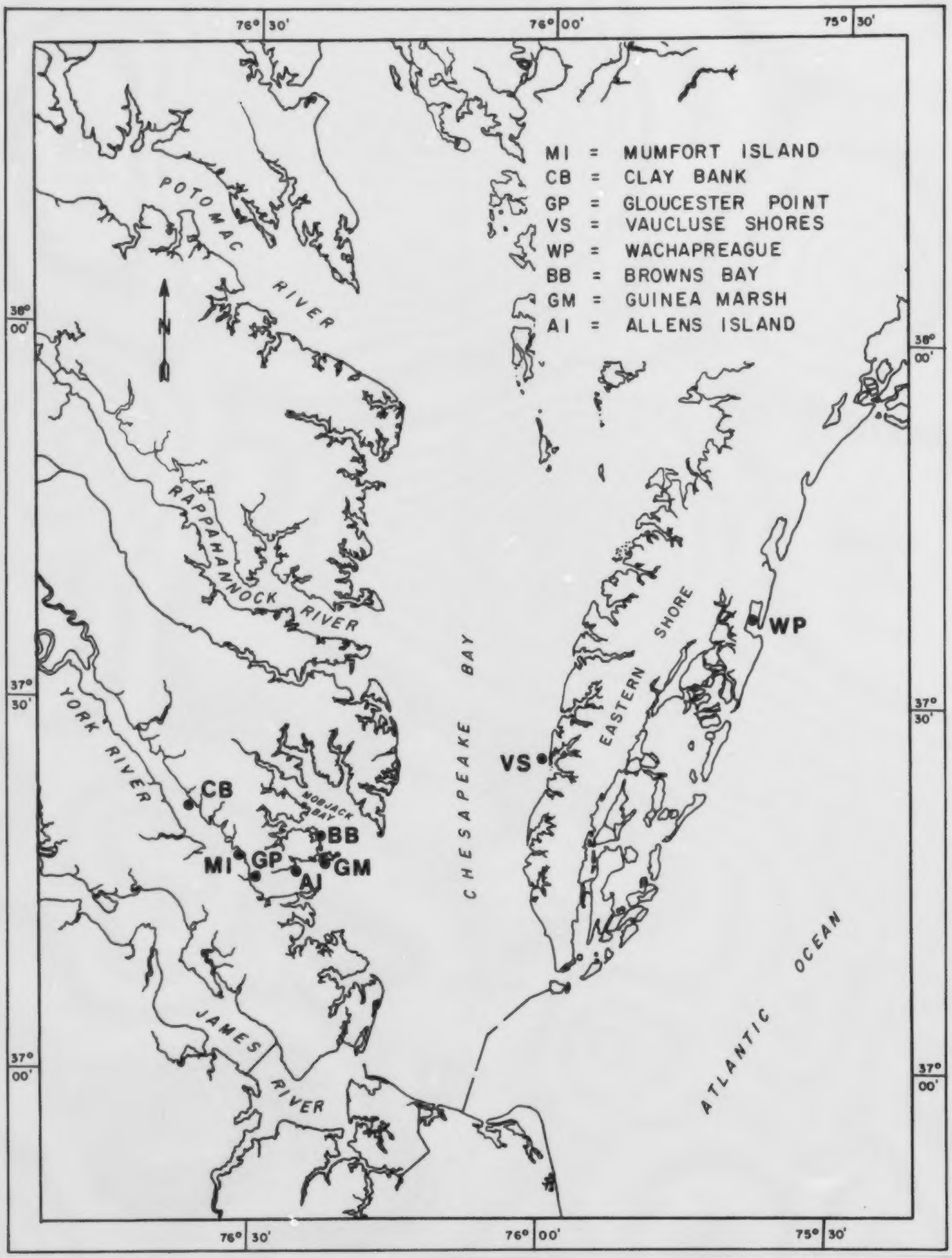
MI = MUMFORT ISLAND
CB = CLAY BANK
GP = GLOUCESTER POINT
VS = VAUCLUSE SHORES
WP = WACHAPREAGUE
BB = BROWNS BAY
GM = GUINEA MARSH
AI = ALLENS ISLAND
76° 30'
76° 00'
75° 30'
38° 00'
37° 30'
37° 00'
N
POTOMAC RIVER
RAPPAHANNOCK RIVER
YORK RIVER
JAMES RIVER
MOBJACK BAY
CHESAPEAKE BAY
EASTERN SHORE
ATLANTIC OCEAN
CB
MI
GP
AI
BB
GM
VS
WP

Fig. 1. Location of study sites used for the standing stock studies. Standing crop measurements made at Vaucluse Shores (VS), Browns Bay (BB) and Guinea Marsh (GM).

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TABLE 1. COMPARISON OF VEGETATIVE AND REPRODUCTIVE DATA COLLECTED USING THE TWO METHODS DESCRIBED IN THE TEXT (0.1 m^2 ring vs. 0.033 m^2 core). TWO SAMPLES WERE COLLECTED WITH EACH METHOD AT TWO DIFFERENT SITES. ALL DATA ARE EXTRAPOLATED TO A PER m^2 BASIS (± 1 STANDARD DEVIATION).

Guinea Marshes 6/28/79

	0.1 m^2 Ring	0.033 m^2 Core
No. of *Zostera* vegetative shoots/ m^2	1330 ± 141	1536 ± 176
Zostera - Mean Length (cm)	42.9 ± 1.7	40.2 ± 3.5
Zostera - Shoot Standing Crop (g/m^2)	301 ± 21	336 ± 48
Zostera - Root and Rhizome Standing Crop (g/m^2)	126 ± 51	130 ± 39
Zostera - Total Biomass (g/m^2)	427 ± 72	467 ± 82
Zostera - Reproductive Shoot Standing Crop (g/m^2)	32 ± 11	21 ± 9

Browns Bay 7/2/79

	0.1 m^2 Ring	0.033 m^2 Core
No. of *Zostera* vegetative shoots/ m^2	2440 ± 113	2433 ± 361
Zostera - Mean Length (cm)	13.8 ± 1.8	19.6 ± 1.8
Zostera - Shoot Standing Crop (g/m^2)	114 ± 35	161 ± 55
Zostera - Root and Rhizome Standing Crop (g/m^2)	154 ± 34	155 ± 52
Zostera - Total Biomass (g/m^2)	268 ± 69	315 ± 103
Ruppia - Mean Length (cm)	12 ± 1.1	13.3 ± 1.4
Ruppia - Total Biomass (g/m^2)	29 ± 16	42 ± 21

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nearest 0.01 g after removing from the dessicator. Parameters recorded included number of vegetative and reproductive shoots per m^2, mean length of shoots, standing crop of the leaf and root and rhizome fractions per m^2,

Temperature and salinity measurements were taken during each sampling trip but more complete temperature data were acquired for a continuously operating temperature sensor located at the Virginia Institute of Marine Science. Although this site was several km from our sampling sites, values for temperature and salinity agreed very closely.

Sediment samples were obtained at each site with small diameter cores to characterize the sediment structure. Sediment samples were processed according to Folk (1961) for silts and clays and dry sieved for sand factors.

RESULTS

Standing Crop Measurements

Zostera marina displayed a striking seasonal growth cycle at all five sampling sites during the approximately two and one-half year, monthly sampling program. Seasonal trends for nunber of shoots, shoot length, shoot standing crop and root-rhizome standing crop were quite similar at the sites even though some slight differences among the sites were evident for each of these parameters. The results for all these measurements are discussed below for each site separately to facilitate easier comprehension of all the data.

Browns Bay

Peak shoot standing crop at this area occurred during the June-July period both in 1979 and 1980 while lowest standing crop was recorded in the fall and winter months (Table 2, Fig. 2). Root and rhizome standing crop followed a similar pattern as the shoot standing crop (Table 2, Fig. 3). Vegetative shoot standing crop averaged 51% of the total biomass of the plant (range of 32-79%) (Fig. 6). Reproductive shoots were present in the spring of 1979 and 1980. Their shoot standing crop accounted for 15 to 32% in 1979 and 11 to 43% in 1980 of the total shoot standing crop (reproductive and vegetative) but 10 to 22% in 1979 and 6 to 24% in 1980 of total biomass which includes the root-rhizome standing crop (Table 2, Fig. 2).

Shoot density was highest also in the June-July period of 1979 while in 1980 there was a peak density in March followed by a decline and then an increase to another maximum of 2333 shoots in June (Table 2, Fig. 4). Shoot density was lowest in the fall period after the summer die-off but began to increase in the early fall beginning around October. Reproductive shoots ranged from 5 to 11% in 1979 to 4 to 20% in 1980 of the total number of shoots (Table 2, Fig. 4).

Mean length of shoots was also highest in the June-July period (19.6 cm in 1979, 15.1 cm in 1980) (Table 2, Fig. 5). The average length of shoots was smallest in March (8.3 cm in 1979, 8.0 cm in 1980) with mean shoot length continually decreasing from the summer maxima through the fall and to the

TABLE 2. MONTHLY GROWTH PARAMETERS FOR ZOSTERA MARINA AND TOTAL BIOMASS (SHOOTS, ROOTS AND RHIZOMES) FOR RUPPIA MARITIMA AT THE BROWNS BAY AREA.

Date	Number of Shoots/ m^2 ± 1 SD	Mean Length (cm) ± 1 SD	Shoot Standing Crop (g/m^2) ± 1 SD	Root and Rhizome Standing Crop (g/m^2) + 1 SD	Total Biomass (g/m^2) ± 1 SD	Ruppia Total Biomass (g/m^2) ± 1 SD
Oct. 23, 1978	788 ± 354	12.0 ± 1.9	23 ± 7	6 ± 2	29 ± 9	11 ± 11
Nov. 30, 1978	870 ± 316	11.4 ± 1.6	24 ± 12	8 ± 7	32 ± 19	14 ± 4
March 7, 1979	835 ± 254	8.3 ± 1.4	11 ± 1	8 ± 2	19 ± 3	8 ± 6
April 5, 1979	1515 ± 449	12.2 ± 1.0	36 ± 6	25 ± 2	72 ± 11	15 ± 12
	138 ± 52*	18.6 ± 1.5*	10 ± 3*		72 ± 11	
May 8, 1979	1500 ± 284	14.3 ± 1.2	82 ± 22	58 ± 21	181 ± 55	14 ± 25
	192 ± 70*	24.3 ± 3.1*	40 ± 17*			
June 6, 1979	2015 ± 774	19.6 ± 2.7	134 ± 40	68 ± 21	224 ± 66	15 ± 11
	115 ± 83*	18.0 ± 9.6*	23 ± 10*			
July 2, 1979	2433 ± 361	16.9 ± 1.8	161 ± 55	155 ± 52	315 ± 103	42 ± 21
July 30, 1979	2576 ± 370	15.1 ± 1.3	152 ± 45	103 ± 30	255 ± 67	21 ± 21
Aug. 30, 1979	1333 ± 200	12.2 ± 1.3	61 ± 15	88 ± 18	148 ± 27	27 ± 18
Sept. 24, 1979	591 ± 191	9.2 ± 1.8	9 ± 3	15 ± 6	27 ± 9	3 ± 3
Oct. 30, 1979	1085 ± 273	9.9 ± 1.2	30 ± 9	27 ± 9	55 ± 15	12 ± 12
Nov. 28, 1979	1524 ± 758	9.6 ± 1.0	52 ± 30	36 ± 30	91 ± 61	12 ± 15
Jan. 22, 1980	2173 ± 352	9.3 ± 0.9	45 ± 6	61 ± 12	106 ± 18	9 ± 6
Feb. 14, 1980	1879 ± 318	9.1 ± 1.1	52 ± 15	76 ± 15	127 ± 24	12 ± 12
March 19, 1980	2918 ± 970	8.0 ± 0.2	48 ± 9	48 ± 12	104 ± 17	9 ± 0
	121 ± 121*	11.8 ± 0.1*	6 ± 6*			
April 21, 1980	1939 ± 318	11.9 ± 3.2	76 ± 39	112 ± 18	223 ± 64	30 ± 12
	203 ± 106*	20.1 ± 1.9*	33 ± 9*			
May 19, 1980	1718 ± 455	13.8 ± 2.3	100 ± 6	136 ± 33	311 ± 38	24 ± 21
	424 ± 170*	20.7 ± 2.3*	76 ± 15*			
June 24, 1980	2333 ± 421	14.0 ± 2.2	173 ± 64	206 ± 55	379 ± 118	21 ± 27
July 28, 1980	1658 ± 364	15.1 ± 0.9	167 ± 52	109 ± 42	273 ± 91	91 ± 61

*represents measurements for reproductive shoots during period of sexual reproduction.

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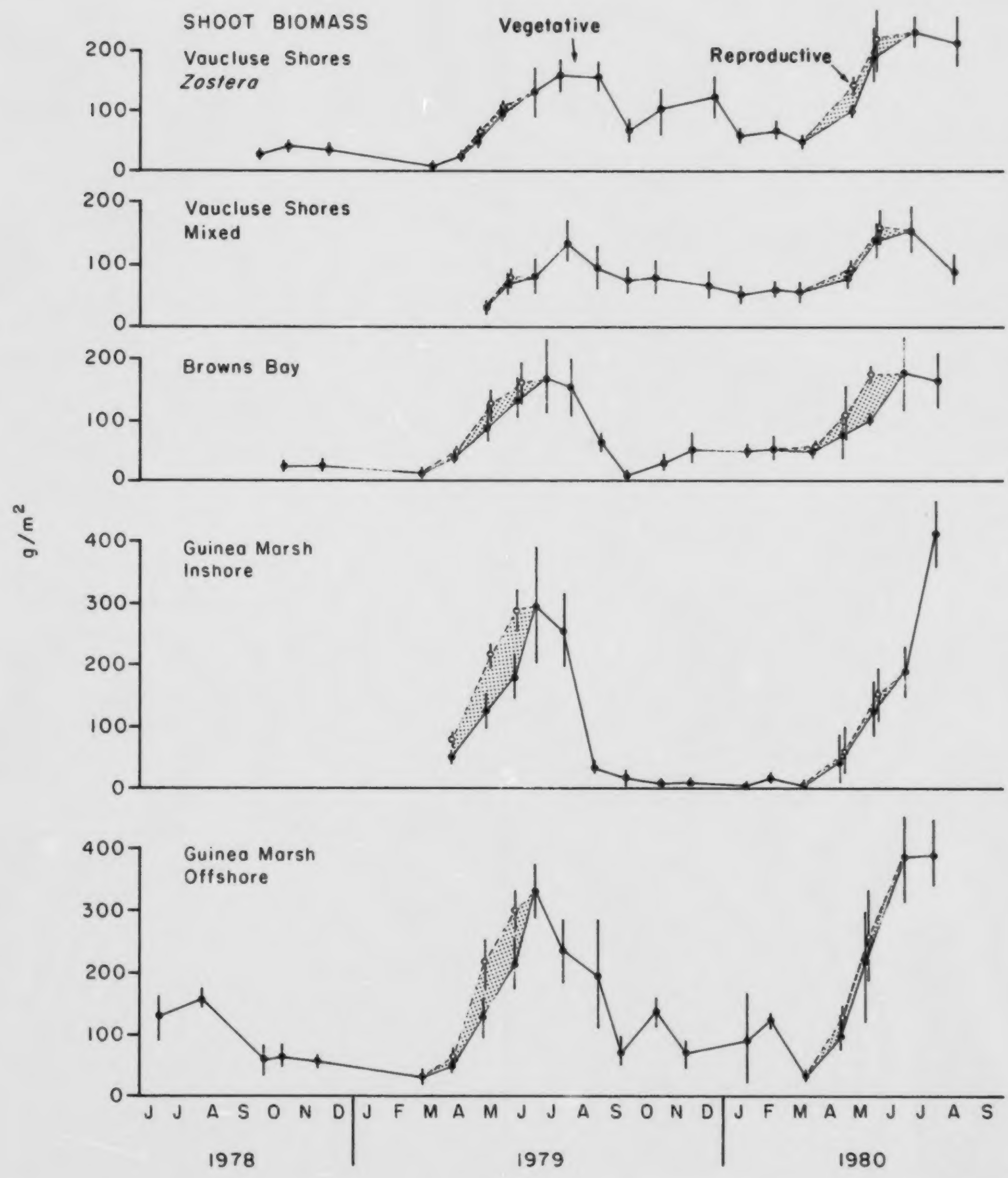

Fig. 2. *Zostera marina* shoot standing crop (g/m^2 ± SD) for each sampling site each season. Difference between open circles-dotted lines and closed circles-solid lines, where they occur, represent additional contributions by reproductive shoots (Stippled).

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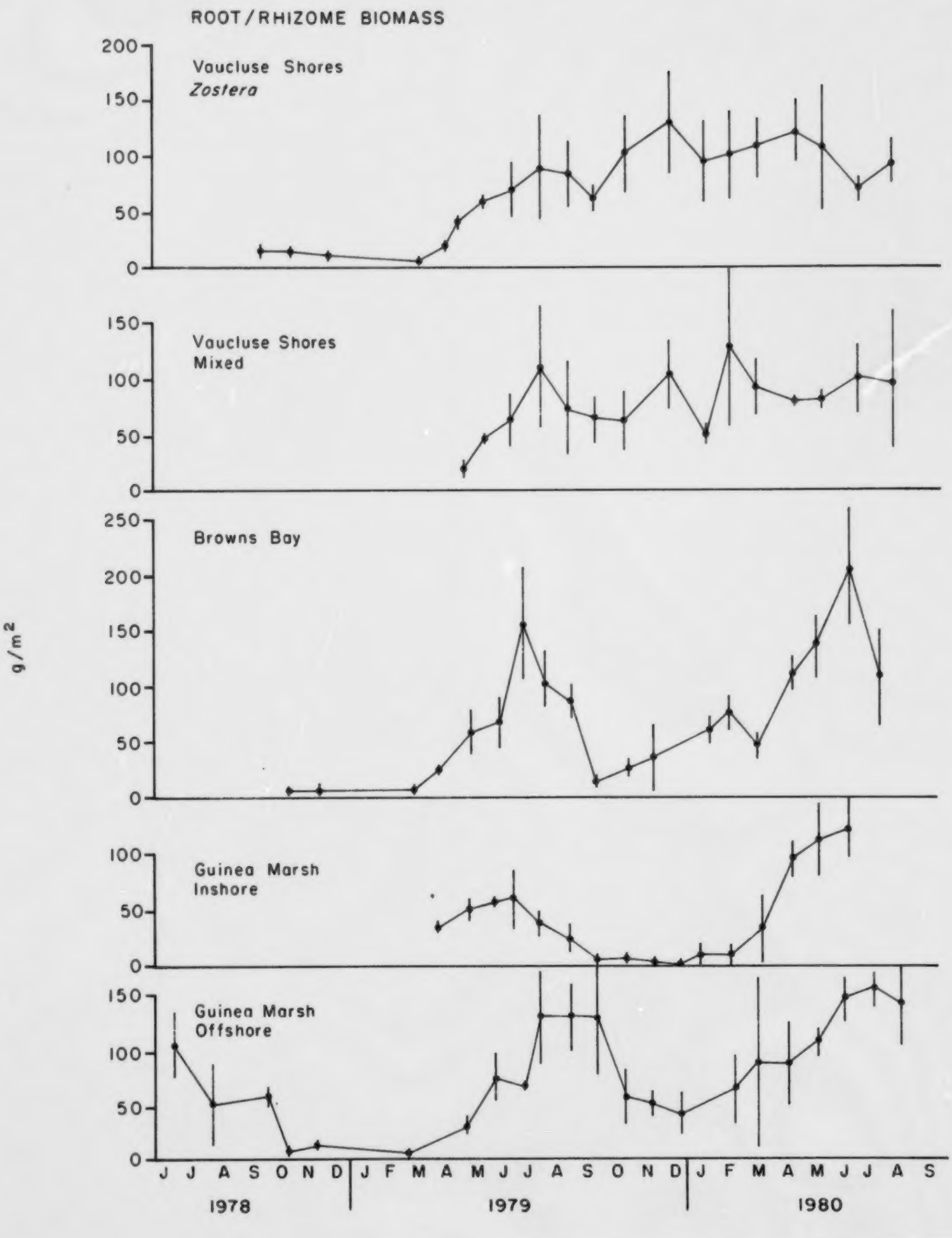

Fig. 3. *Zostera marina* root and rhizome standing crop (g/m^2 ± 1 SD) for each site each sampling period.

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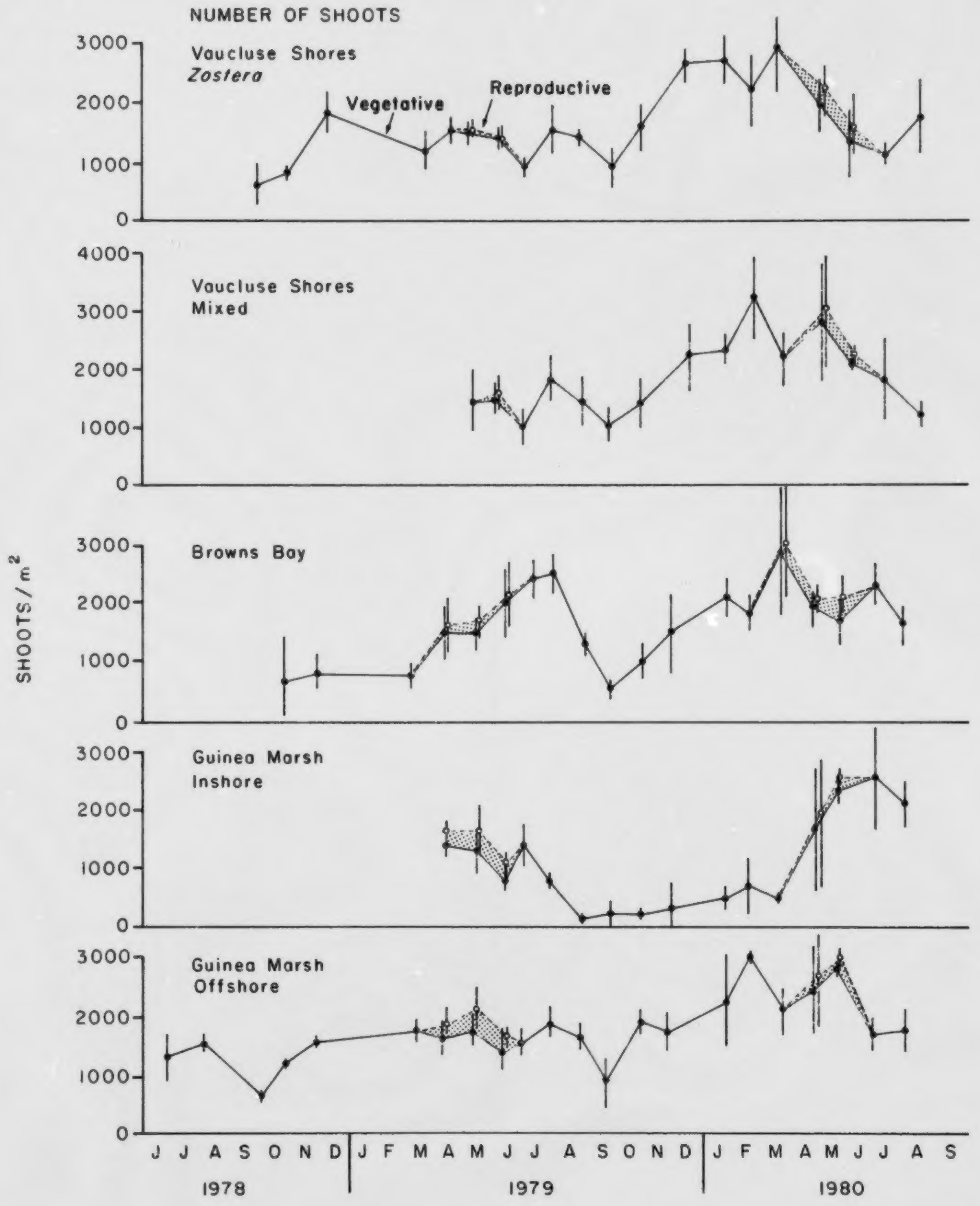

Fig. 4. Number of *Zostera marina* shoots per m^2 (± 1 SD) for each sampling site each sampling period. Difference between open circles, dotted lines and closed circles solid lines, where they occur, represents contribution by reproductive shoots (stippled area).

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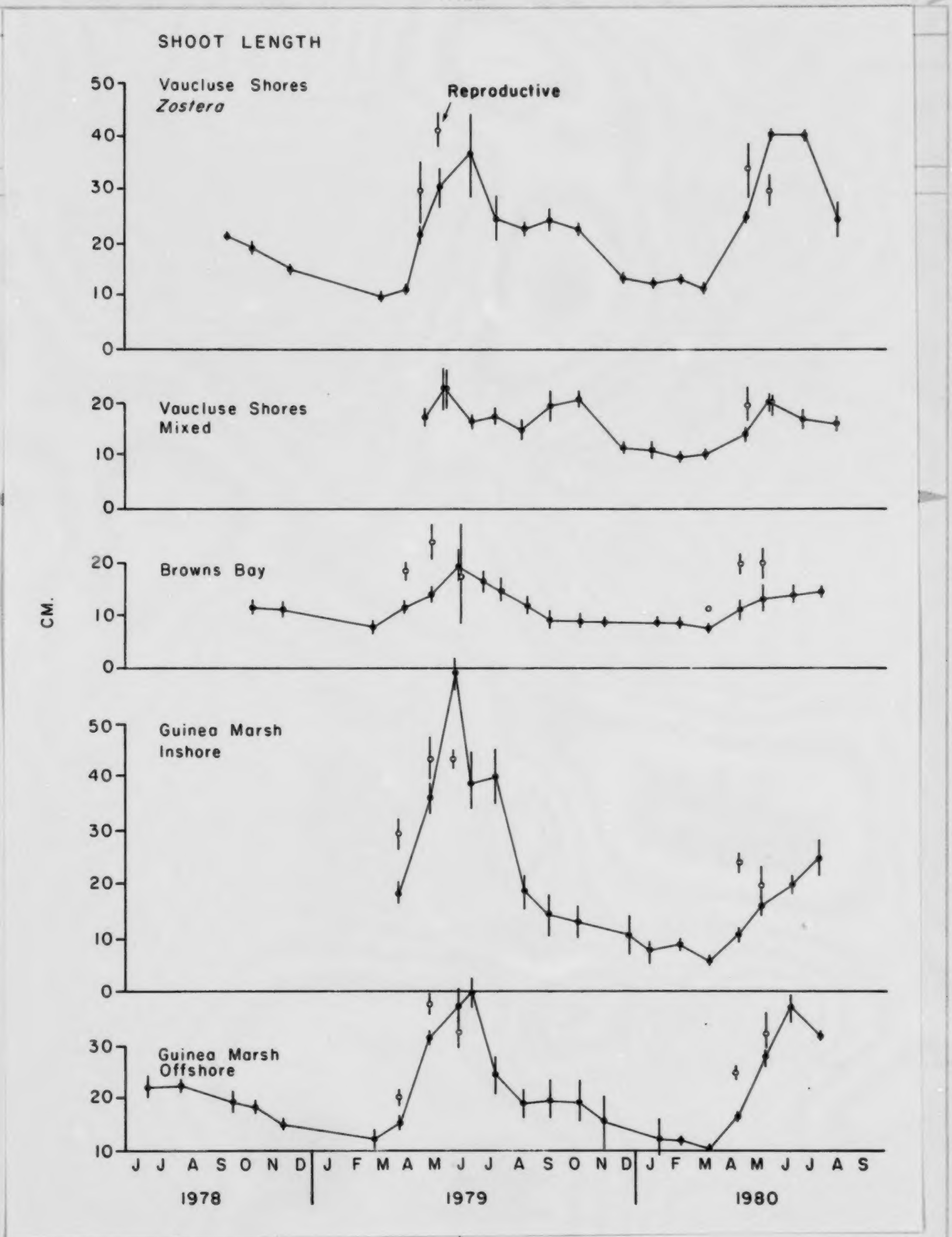

Fig. 5. Shoot length (cm) (± 1 SD) for vegetative shoots of *Zostera marina* during each sampling period for each site. Open circles represent length of reproductive shoots for the sampling period.

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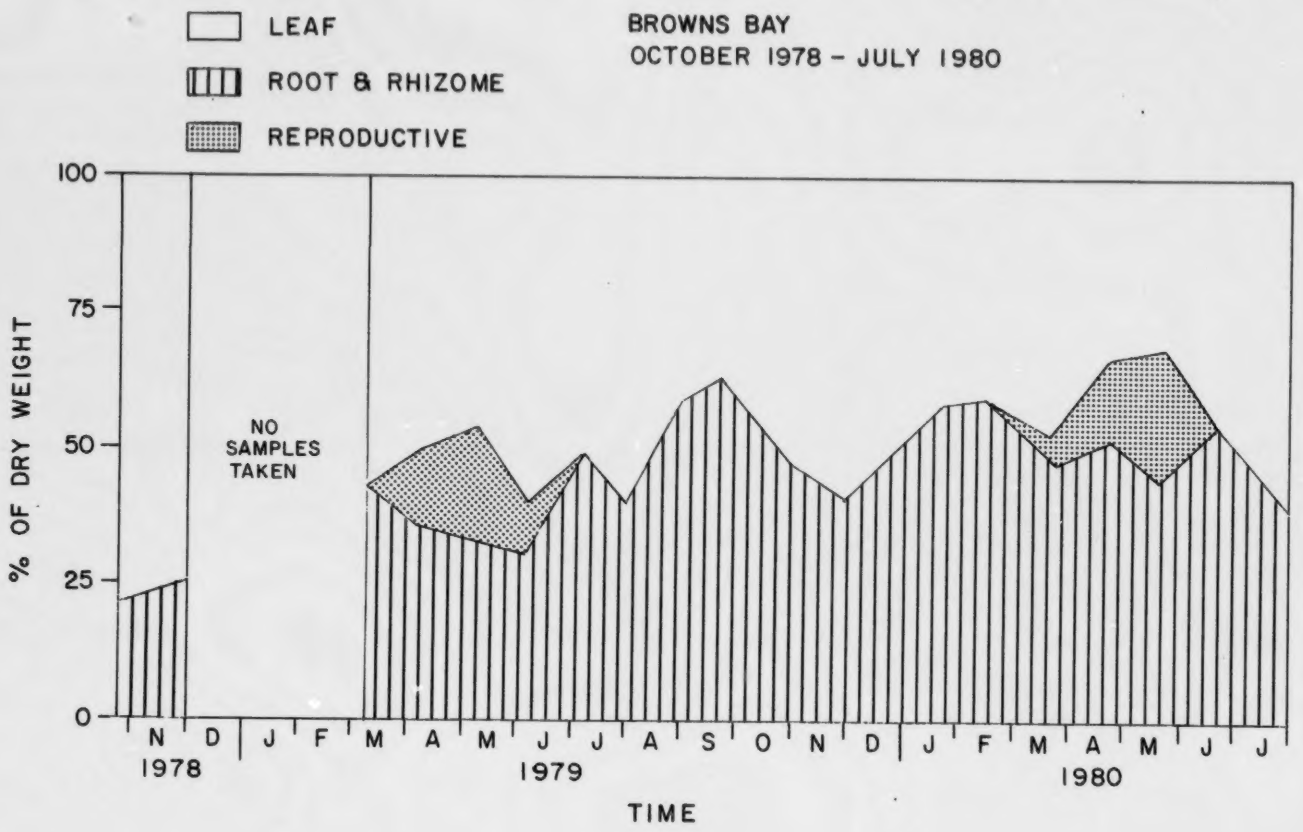

Fig. 6. Partitioning of total biomass of Zostera marina into leaf, root and rhizome and reproductive fractions based on percent dry weight for Z. marina at the Browns Bay site. (see Table 2 for raw data).

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early spring (March) period when shoot length again begins to increase. Examination of frequency histograms of the different size classes (5 cm intervals) shows the seasonal pattern inchanges in the percent of number of shoots in each size category (Fig. 7). Several patterns are evident from these histograms. First, the percentage of shoots in the larger size classes increases from the winter period, when shoots are less than 25 cm, to the summer period, when some shoots have leaves greater than 35 cm. Second, the number of size classes with shoots present is greater in the summer than in the winter (9 vs 4). Third, the percentage of shoots in the smallest size class (0.4 cm) is lowest in the summer (in some momths, there are no small shoots), with new, small shoots being observed for the first time since mid-summer in September. Reproductive shoots, when present, were always longer than the vegetated shoots except at the end of the reproductive period as the reproductive shoots decayed (Table 2, Fig. 5).

Ruppia maritima was present with *Zostera marina* at the Browns Bay area. Examination of total biomass figures for *R. maritima* (Table 2) indicated that maximum biomass occurred during July in both 1979 and 1980 with lowest biomass occurring in the fall and winter months.

Guinea Marsh Offshore

Peak shoot standing crop in this area occurred in the June-July period in both 1979 and 1980 while lowest standing crop occurred in March of both years (Table 3, Fig. 2). Root-rhizome weights peaked also during the same period as shoot standing crop but minimum values were different during the course of study (Table 3, Fig. 3). Low values were 10 g/m^2 in October 1978, and March 1979, while the lowest values during the fall and winter of 1979 and 1980 were 42 g/m^2 in November 1979, and 88 g/m^2 in February and March 1980, four to eight times higher than the previous year. Leaf biomass averaged 62% of the total biomass of the plant (range 27 to 86%) during the course of the study (Fig. 8). Reproductive shoots were present in the spring of both years. Their standing crop of shoots accounted for 24 to 41% in 1979 and 14 to 24% in 1980 of total shoot biomass but 16 to 31% in 1979 and 9 to 13% in 1980 of total biomass (Table 3, Figs. 2 & 8).

The seasonal pattern for shoot density in this area was not as clear cut as in the Browns Bay area (Table 3, Fig 4). Density was lowest in the fall (September-October) in both 1978 and 1979. New shoot production increased rapidly (e.g. 695 to 1233 shoots/m^2 from October 3, 1978 to October 23, 1978) with highest shoot density occurring during periods other than the June-July period. There were very high density of shoots in January and February 1980, which remained high until June-July when recorded shoot density had decreased. Reproductive shoots constituted 12 to 18% in 1979 and 6 to 11% in 1980 of the total number of shoots in the area (Table 3, Fig. 4).

Shoot length was highest in the June period of 1979 and 1980, decreasing through the fall and winter and reaching minimal shoot length by March (Table 3, Fig. 5). This pattern was similar to Browns Bay, which was a result of the same events discussed for Browns Bay. The large number of small shoots in March of both years (Fig. 9) resulted in the low mean length. Subsequent increase in length of these shoots as evidenced by the shift in percent of

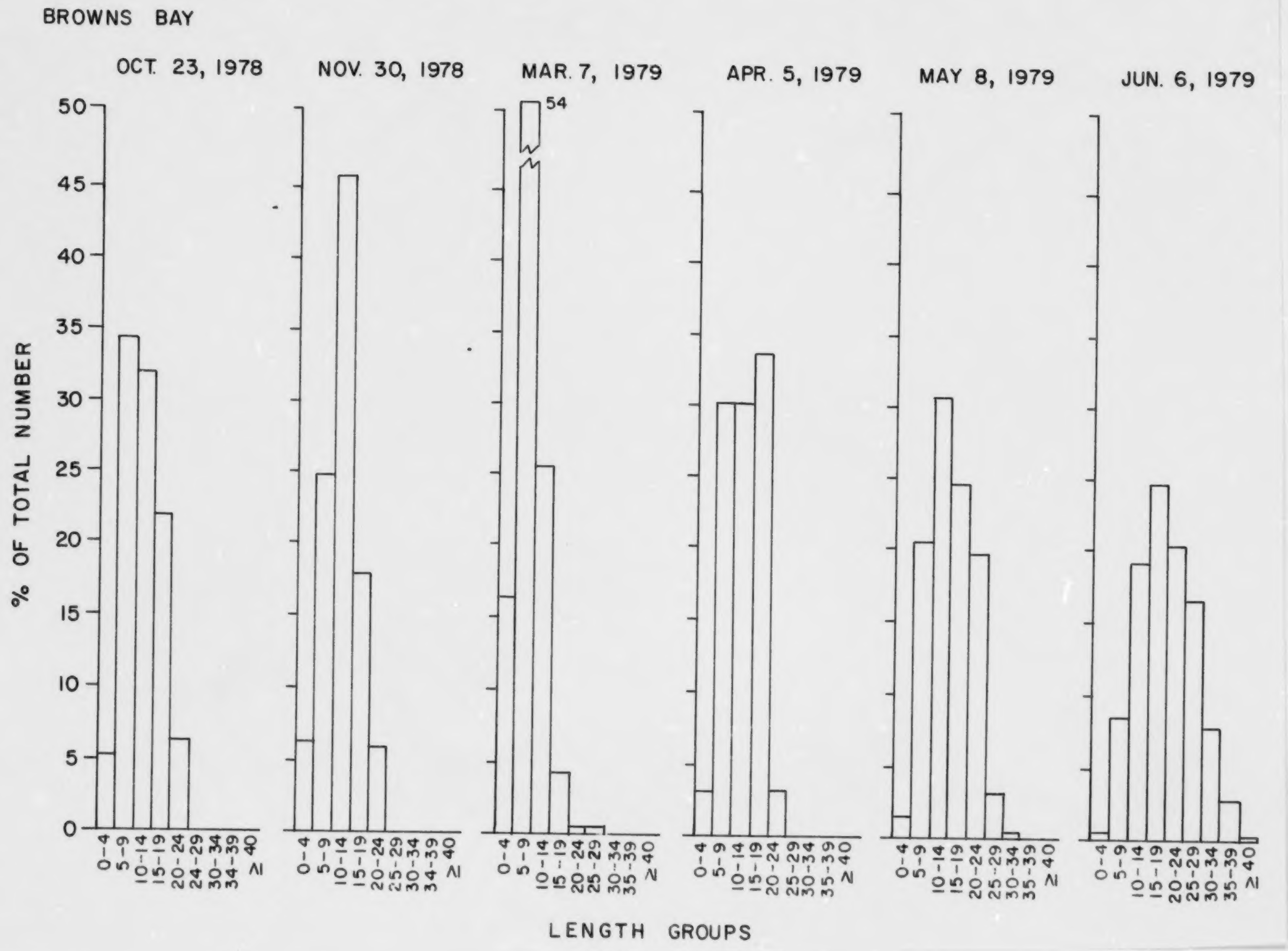
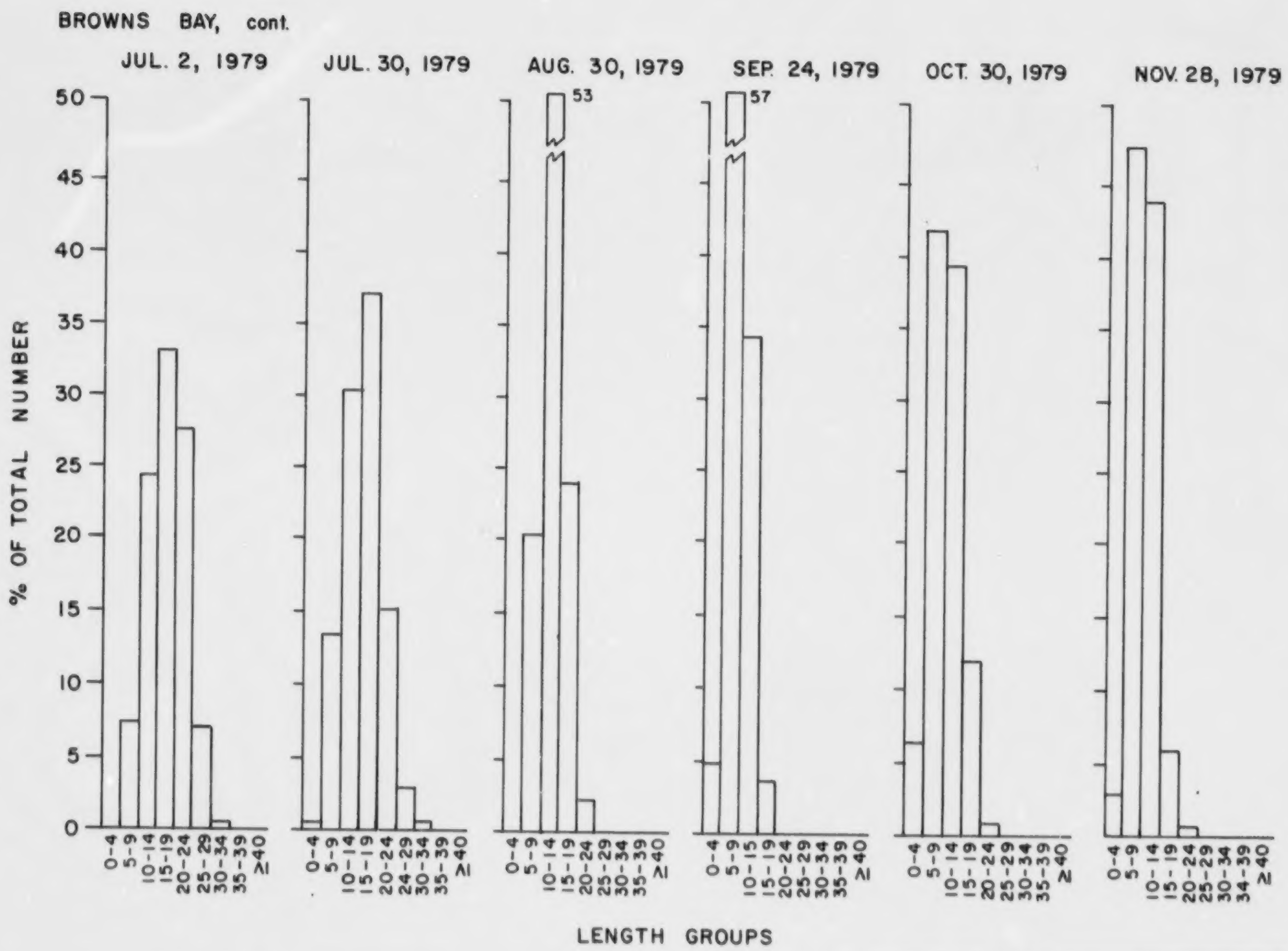


Fig. 7. Frequency histograms of the percent of vegetative shoots in the different size class categories for all sampling periods for Zostera marina at Browns Bay.



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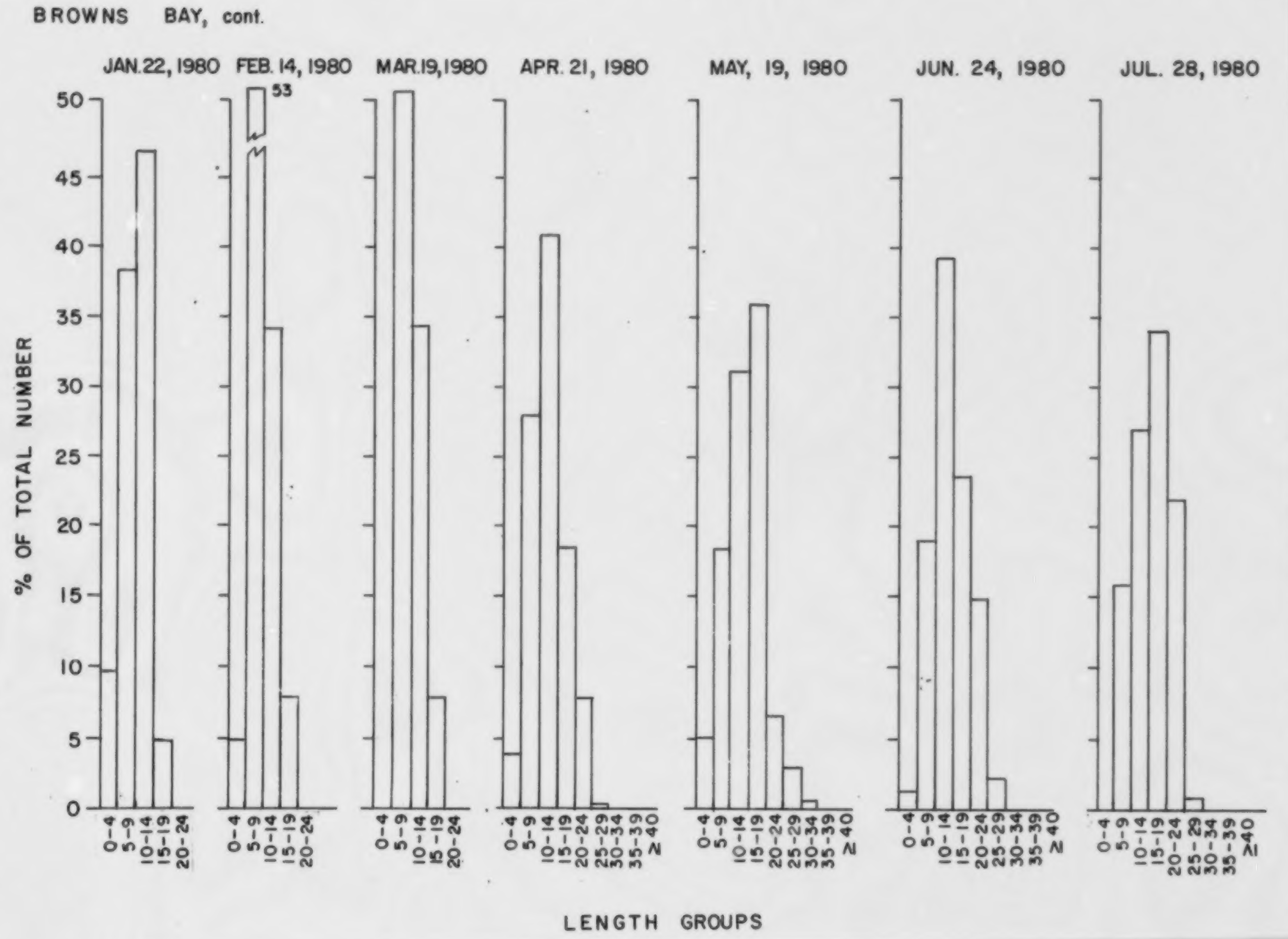

Fig. 7 (continued)

TABLE 3. MONTHLY GROWTH PARAMETERS FOR ZOSTERA MARINA AT THE GUINEA MARSHES OFFSHORE AREA.

Date	Number of Shoots / m^2 ± 1 Standard Deviation	Mean Length (cm) ± 1 Standard Deviation	Shoot Standing Crop (g/m^2) ± 1 Standard Deviation	Root and Rhizome Standing Crop (g/m^2) ± 1 Standard Deviation	Total Biomass (g/m^2) ± 1 Standard Deviation
June 28, 1978	1330 ± 456	22.6 ± 2.0	132 ± 39	105 ± 29	237 ± 64
Aug. 3, 1978	1578 ± 140	22.8 ± 1.0	158 ± 12	50 ± 3	208 ± 41
Oct. 3, 1978	695 ± 107	19.2 ± 2.0	57 ± 20	26 ± 10	83 ± 29
Oct. 23, 1978	1233 ± 83	18.5 ± 1.7	63 ± 17	10 ± 4	73 ± 17
Nov. 30, 1978	1538 ± 74	15.6 ± 1.0	54 ± 4	14 ± 2	68 ± 2
March 7, 1979	1850 ± 188	12.4 ± 2.0	34 ± 8	10 ± 1	44 ± 9
April 3, 1979	1690 ± 243	15.4 ± 1.1	51 ± 10	34 ± 8	101 ± 5
	240 ± 78*	20.3 ± 1.5*	16 ± 3*		
May 8, 1979	1805 ± 336	31.3 ± 1.4	130 ± 22	75 ± 24	294 ± 21
	365 ± 165*	37.9 ± 1.6*	90 ± 37*		
June 6, 1979	1418 ± 202	37.7 ± 3.5	216 ± 25	68 ± 3	373 ± 43
	308 ± 28*	32.5 ± 3.4*	89 ± 19*		
June 28, 1979	1536 ± 176	40.2 ± 3.5	336 ± 48	130 ± 39	467 ± 82
July 26, 1979	1903 ± 252	24.0 ± 3.9	239 ± 55	130 ± 30	370 ± 82
Aug. 28, 1979	1691 ± 473	19.0 ± 2.1	197 ± 88	127 ± 45	324 ± 124
Sept. 24, 1979	909 ± 164	19.9 ± 3.7	73 ± 24	58 ± 21	130 ± 39
Oct. 30, 1979	1970 ± 300	19.8 ± 3.8	145 ± 15	52 ± 12	197 ± 27
Nov. 28, 1979	1718 ± 882	15.9 ± 5.1	70 ± 21	42 ± 21	112 ± 39
Jan. 22, 1980	2364 ± 891	12.9 ± 3.0	94 ± 73	67 ± 33	161 ± 106
Feb. 14, 1980	3030 ± 79	12.2 ± 0.7	127 ± 9	88 ± 73	215 ± 82
March 19, 1980	2161 ± 312	10.8 ± 0.5	33 ± 3	88 ± 42	121 ± 42
April 21, 1980	2424 ± 730	16.5 ± 0.8	97 ± 21	109 ± 15	236 ± 48
	303 ± 133*	24.9 ± 1.2*	30 ± 12*		

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TABLE 3. (continued)

Date	Number of Shoots / m^2 ± 1 Standard Deviation	Mean Length (cm) ± 1 Standard Deviation	Shoot Standing Crop (g/m^2) ± 1 Standard Deviation	Root and Rhizome Standing Crop (g/m^2) ± 1 Standard Deviation	Total Biomass (g/m^2) ± 1 Standard Deviation
May 19, 1980	2870 ± 121	27.4 ± 1.5	224 ± 82	145 ± 18	403 ± 84
	173 ± 127*	32.1 ± 4.7*	36 ± 18*		
June 24, 1980	1718 ± 215	37.5 ± 2.9	394 ± 73	155 ± 18	548 ± 82
July 28, 1980	1767 ± 288	32.0 ± 0.6	397 ± 67	139 ± 48	536 ± 115

*represents measurements for reproductive shoots during period of sexual reproduction.

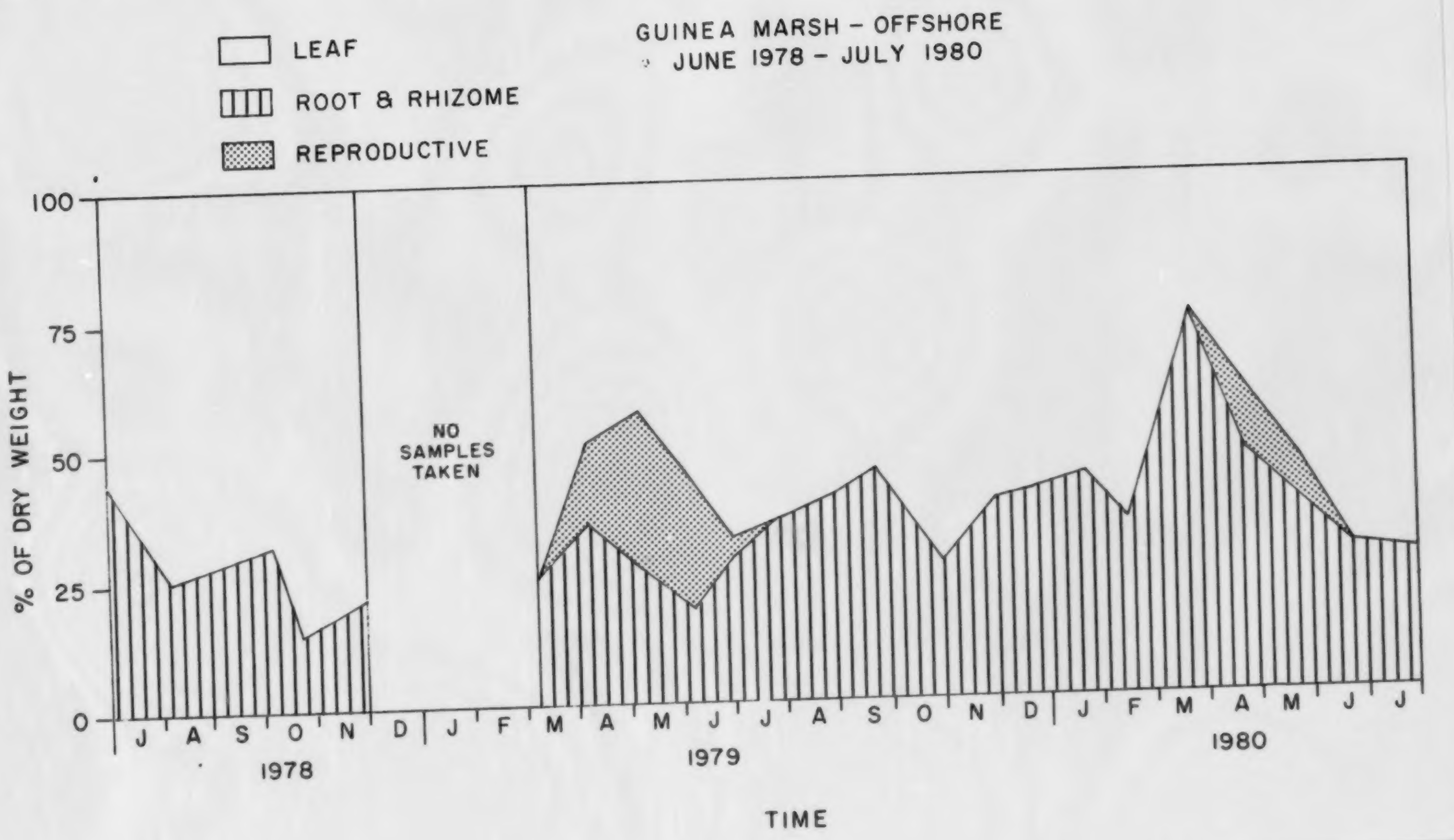


Fig. 8. Partitioning of total biomass of Zostera marina into leaf, root and rhizome and reproductive fractions based on percent dry weight for the Guinea Marsh, Offshore area (see Table 3 for raw data).

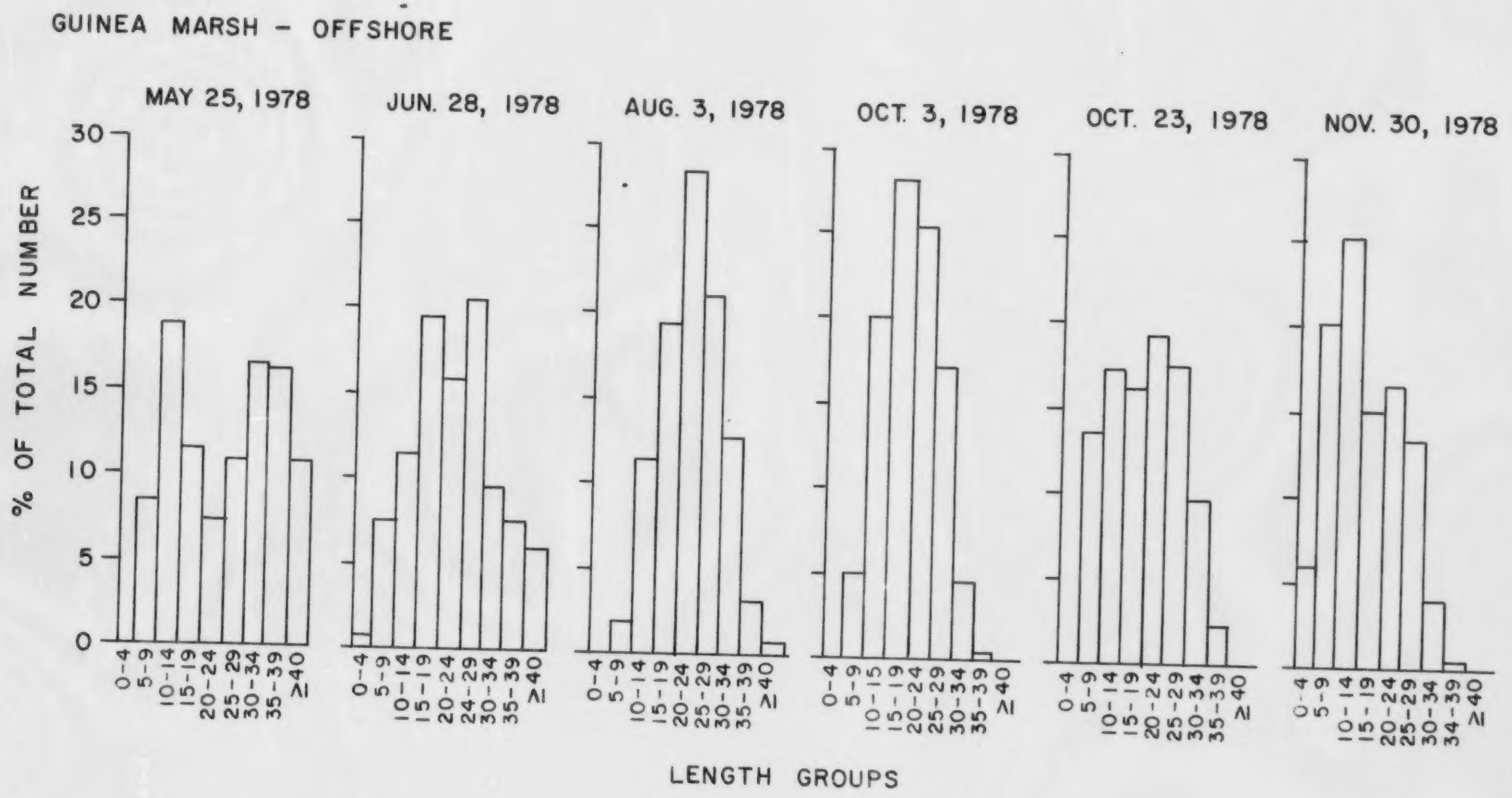
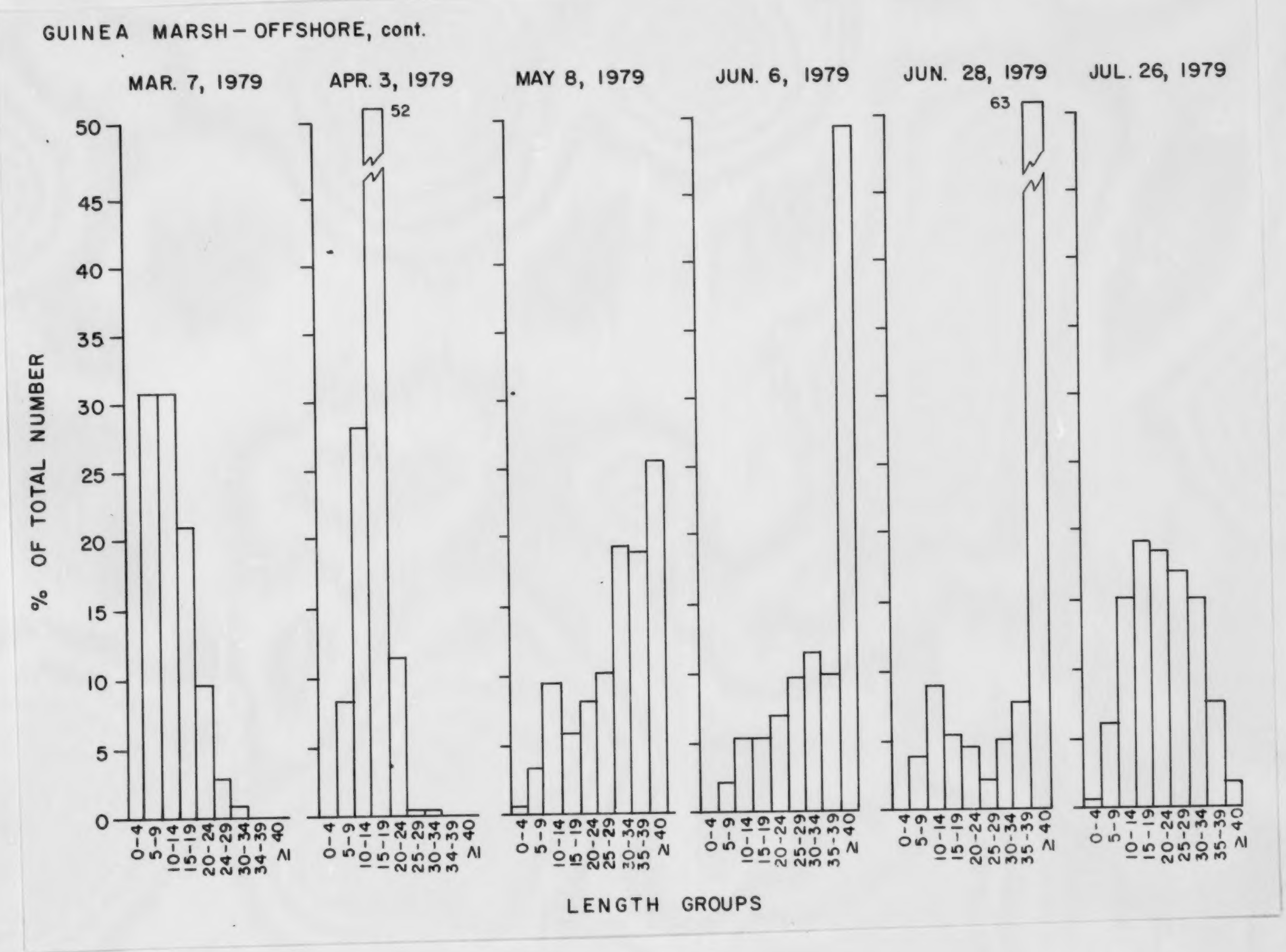


Fig. 9. Frequency histograms of the percent of vegetative shoots in the different size class categories for all sampling periods for Zostera marina at the Guinea Marsh Offshore area.



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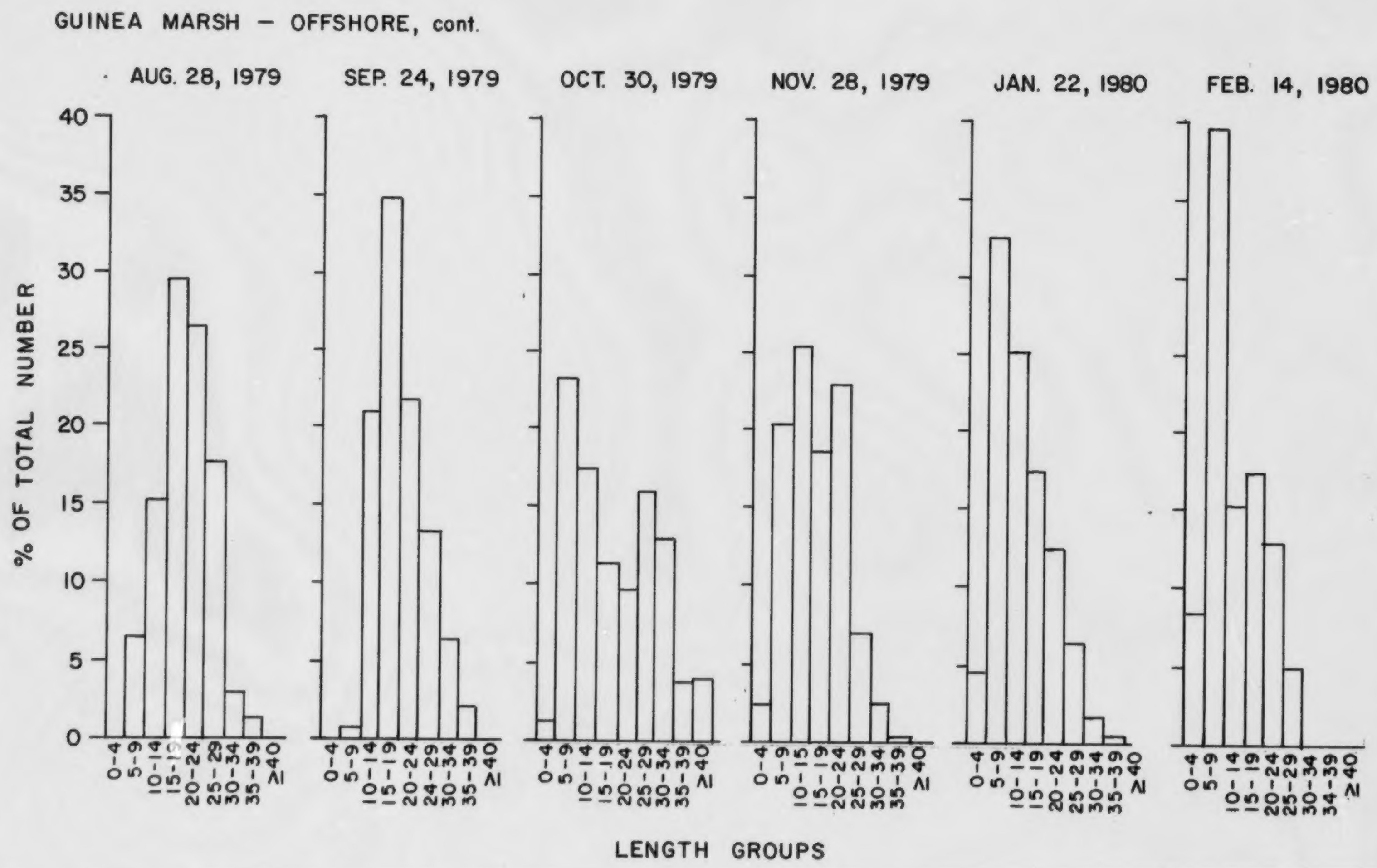

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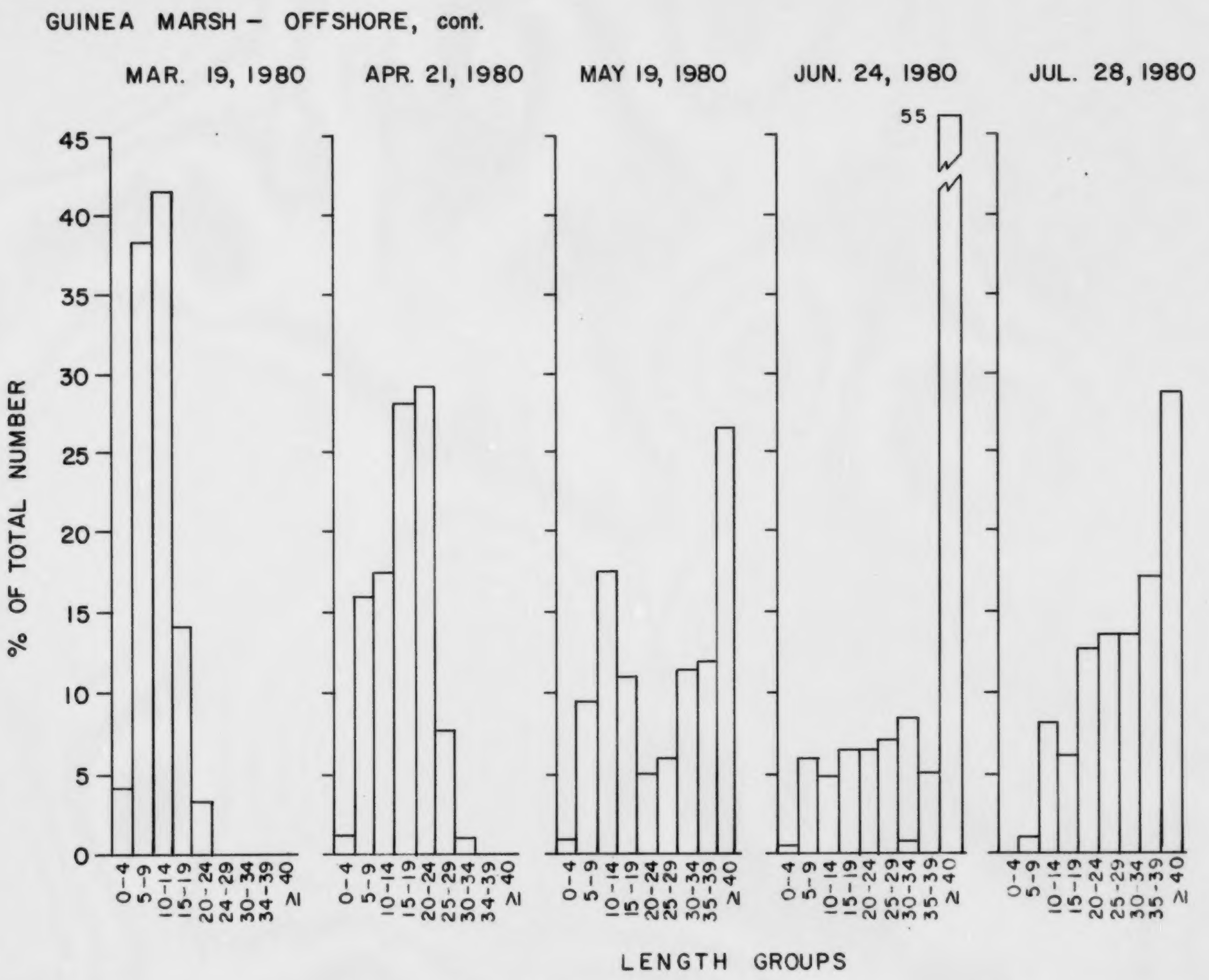


Fig. 9 (continued)

each size category with larger size categories increasing from March to June, caused the large increase in total mean length.

Reproductive shoots were longer than vegetative shoots during most of the reproductive period (Table 3, Fig. 5).

Guinea Marsh Inshore

The seasonal aspects of standing stock measurements in this area were very interesting because of the large difference that were observed between 1979 and 1980 for all measured parameters.

Peak shoot standing crop occurred during the June-July period in both years (Table 4, Fig. 2) but there was a large difference between years. The maximum standing crop in 1979 was 291 g/m^2 but was 412 g/m^2 in 1980. The standing stock decreased dramatically in 1979 to a low of only 2 g/m^2 in January 1980, a sharp drop not observed in the other areas. Shoot standing crop averaged 61% of total biomass (range 40 to 80%) for the sampling period (Fig. 10). Differences in root and rhizome standing crop were just as dramatic: a maximum of 121 g/m^2 in 1980 vs. 61 g/m^2 in 1979 with a low of 1 g/m^2 recorded in January 1980 (Table 4, Fig. 3). Standing crop of reproductive shoots were much higher in 1979 (31 to 42% of total shoot standing crop) compared to 1980 (10 to 17% of total shoot biomass) (Table 4, Figs. 3 & 10).

Shoot density was high through the spring and summer of 1979 but then declined rapidly between June and August 1979, going from 1418 $shoots/m^2$ to 206 $shoots/m^2$ (Table 4, Fig. 4). Compared to the other areas, shoot density did not increase dramatically in the fall but remained low until the spring of 1980. Shoot density increased very rapidly from $515/m^2$ in March to $2597/m^2$ in June. Reproductive shoot density also differed reamarkably from 14 to 25% of total shoots in 1979 to 3 to 4% of total shoots in 1980.

Shoot length was highest in June-July of both years but the mean length of plants in 1979 was much greater than those in 1980 (43.9 cm in 1979 vs 25.5 cm in 1980).

The cause for the big difference between the two years was a major loss of shoots in 1979 (Fig. 11). This area changed dramatically from a lush, dense bed of long vegetative shoots, in which 61% of the shoots were greater than 40 cm, to a barren area of few, shorter shoots. Recovery of this area did not come from vegetative processes (the reason why there was not a great increase in number of shoots in the fall) but from seedling recruitment. We observed large numbers of germinated seedlings in this area (up to 66 m^2) beginning in November 1979 and continuing through March 1980 (germination of eelgrass seeds occurs during this period; see section in this report on seed germination). The production from the abundant seedlings caused shoot density to increase and thus standing crop of shoots, but the mean length of these newer shoots was low, thus causing the differences between the years for shoot density, length and standing crop. Because reproductive shoots are not formed until the second year after a seed germinates (Setchell, 1929) and since most shoots were seedlings, this accounted for the low percentage of

TABLE 4. MONTHLY GROWTH PARAMETERS FOR ZOSTERA MARINA AT THE GUINEA MARSH INSHORE AREA.

Date	Number of Shoots/ m^2 ± 1 SD	Mean Length (cm) ± 1 SD	Shoot Standing Crop (g/m^2)	Root and Rhizome Standing Crop (g/m^2) ± 1 SD	Total Biomass (g/m^2) ± 1 SD
April 3, 1979	1438 ± 266	18.5 ± 1.3	56 ± 11	35 ± 5	117 ± 18
	225 ± 25*	29.6 ± 2.3*	26 ± 4*		
May 8, 1979	1345 ± 441	36.4 ± 3.5	123 ± 21	50 ± 10	263 ± 34
	368 ± 51*	43.7 ± 3.6*	90 ± 10*		
June 5, 1979	835 ± 161	59.7 ± 3.3	187 ± 30	59 ± 2	346 ± 9
	285 ± 60*	43.9 ± 1.7*	100 ± 35*		
June 28, 1979	1418 ± 330	39.1 ± 5.6	291 ± 91	61 ± 27	352 ± 118
July 26, 1979	870 ± 112	40.4 ± 5.0	255 ± 58	36 ± 12	288 ± 70
August 28, 1979	206 ± 45	19.2 ± 2.9	33 ± 12	24 ± 12	57 ± 21
Sept. 24, 1979	327 ± 215	14.6 ± 3.6	18 ± 15	6 ± 3	27 ± 18
Oct. 30, 1979	327 ± 97	13.4 ± 2.1	9 ± 3	6 ± 3	15 ± 3
Nov. 28, 1979	388 ± 394	10.7 ± 2.6	9 ± 9	3 ± 3	11 ± 12
Jan. 22, 1980	515 ± 170	8.0 ± 2.0	2 ± 2	1 ± 0	4 ± 1
Feb. 14, 1980	706 ± 585	9.2 ± 1.2	18 ± 9	9 ± 9	27 ± 18
March 19, 1980	515 ± 9	5.9 ± 0.6	6 ± 0	9 ± 9	15 ± 9
April 21, 1980	1736 ± 1018	10.4 ± 1.6	45 ± 36	33 ± 30	90 ± 71
	70 ± 76*	24.2 ± 1.8*	9 ± 9*		
May 19, 1980	2333 ± 209	16.5 ± 2.4	133 ± 36	94 ± 18	243 ± 54
	82 ± 94*	19.8 ± 4.0*	15 ± 24*		
June 24, 1980	2597 ± 855	19.4 ± 2.0	197 ± 33	112 ± 27	309 ± 58
July 28, 1980	2130 ± 403	25.5 ± 3.9	412 ± 42	121 ± 30	536 ± 42

*represents measurements for reproductive shoots during period of sexual reproduction.

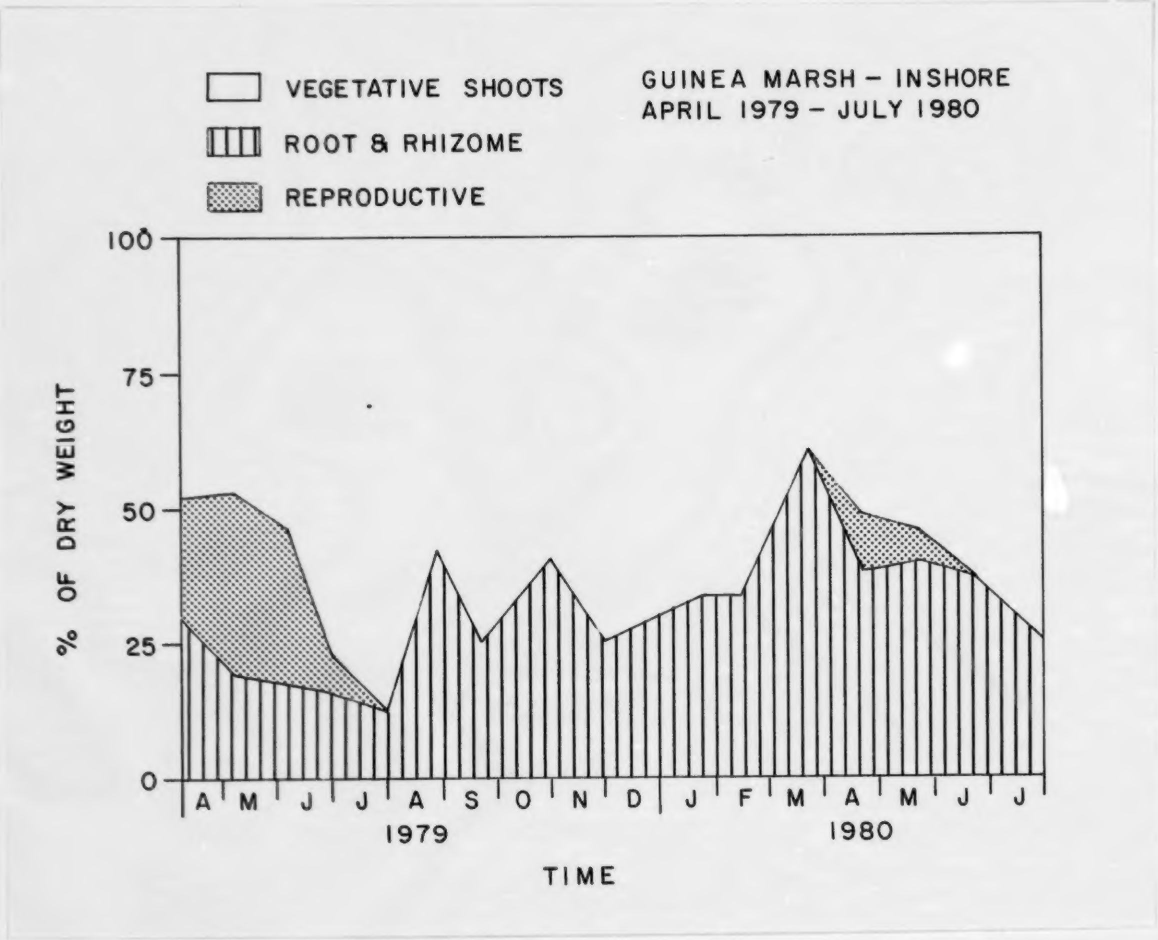


Fig. 10. Partitioning of total biomass of Zostera marina into leaf, root and rhizome and reproductive fractions based on percent dry weight for the Guinea Marsh Inshore area (see Table 4 for raw data).

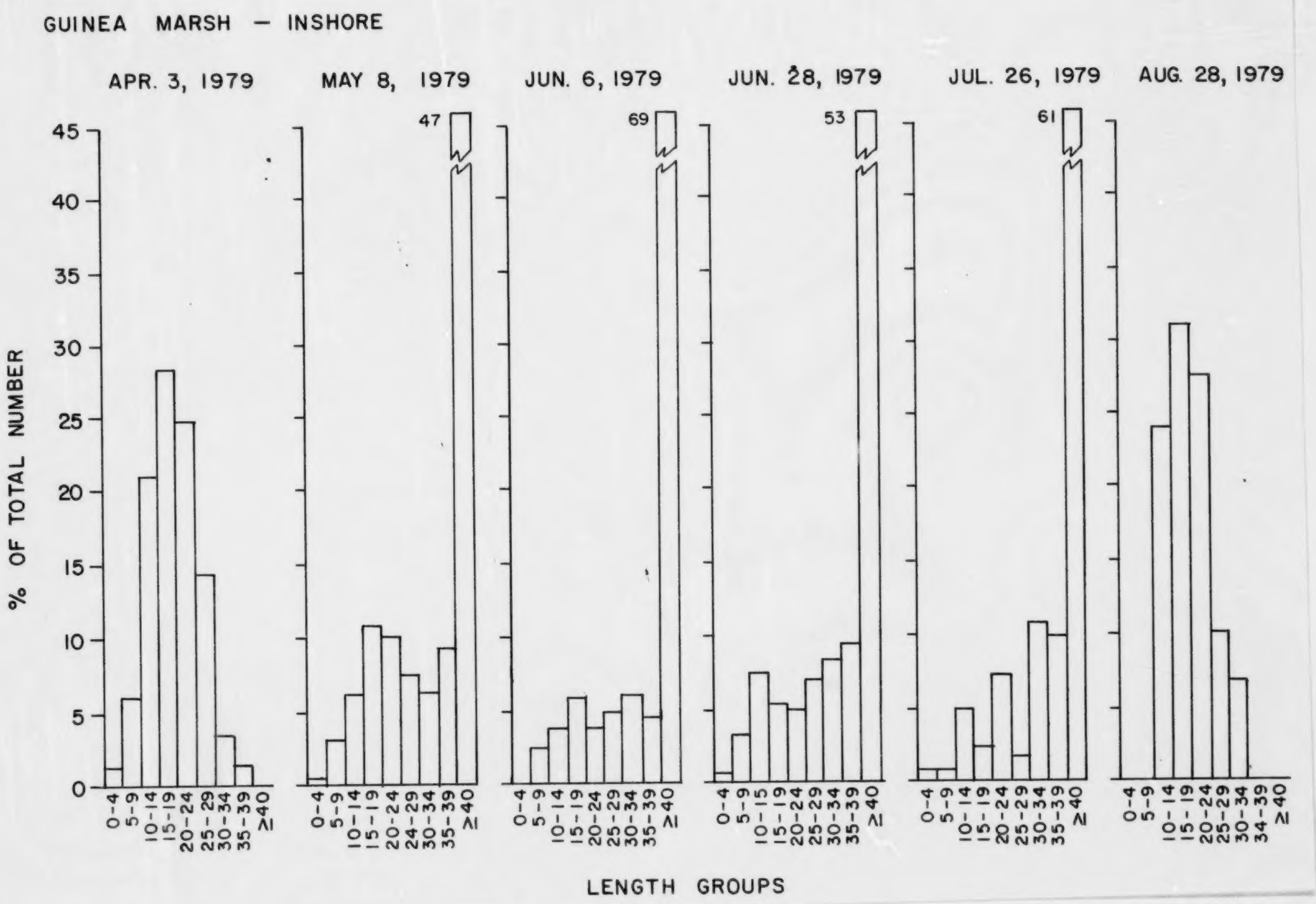
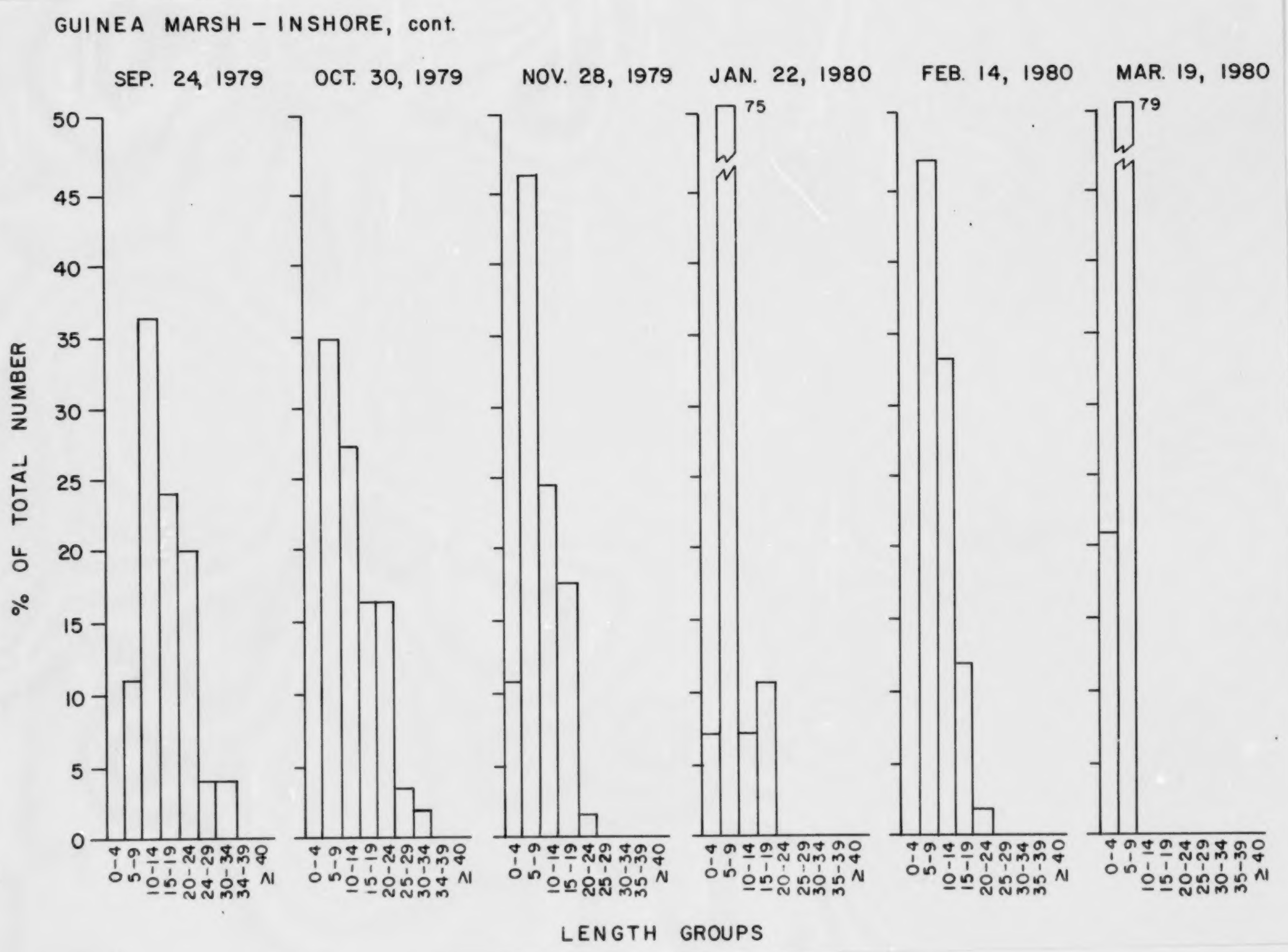


Fig. 11. Frequency histograms of the percent of vegetative shoots in the different size class categories for all sampling periods for Zostera marina at the Guinea Marsh Inshore area.



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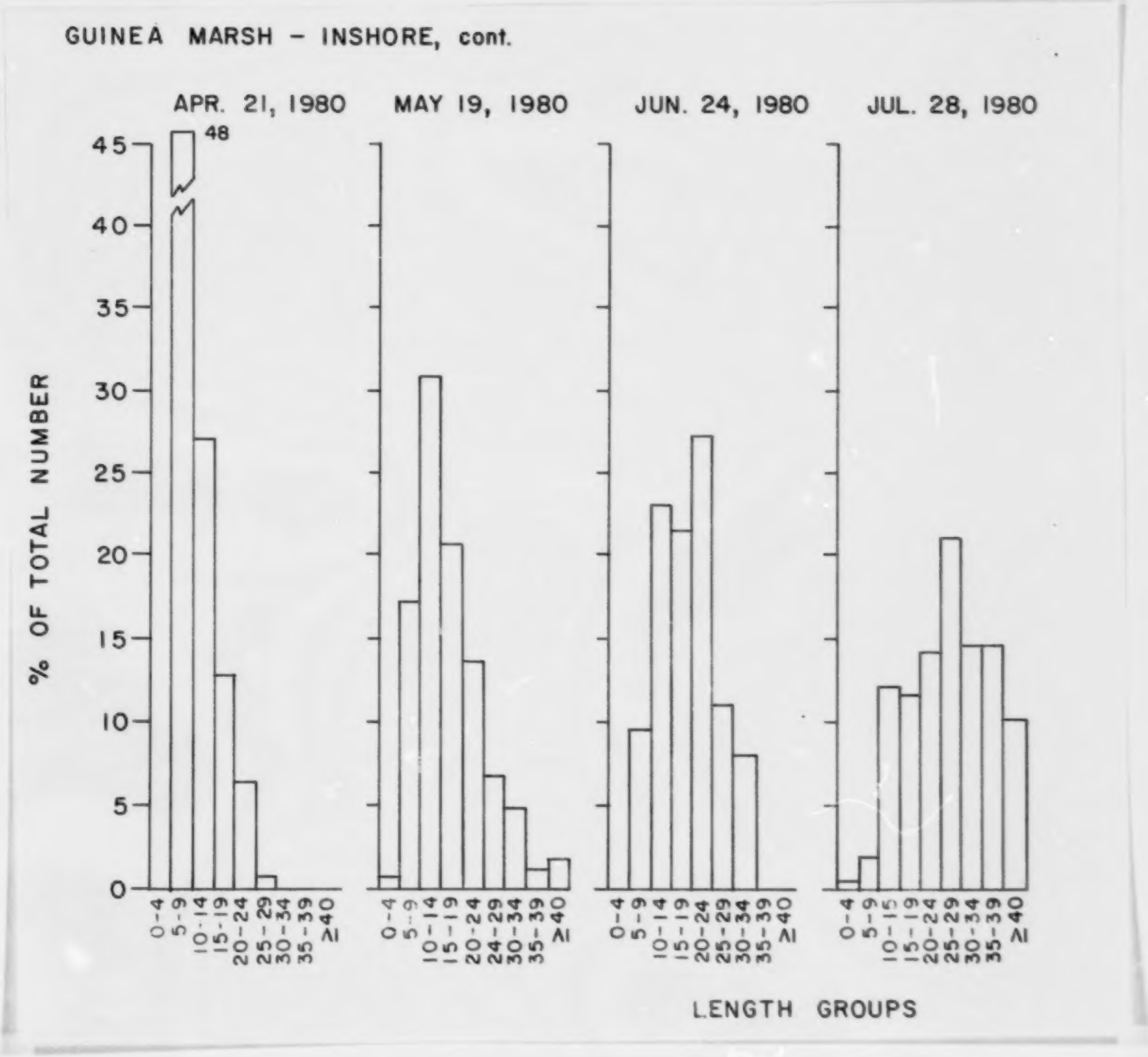

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reproductive shoots in 1980 compared to 1979. The pulse of small shoots produced by the new seedlings in 1980 are shown in the frequency histograms, especially for January through March, 1980 (Fig. 11).

Vaucluse Shores - *Zostera* Bed

Peak shoot standing crop in this area occurred in the June-July period (Table 5, Fig. 2) in both years while lowest standing crop figures were found in March of each year, though estimates were also low in the fall period. Shoot standing crop averaged 58% (range of 33 to 37%) of total biomass of the plant during the study period (Fig. 12). Standing crop estimates for roots and rhizomes presented a dissimilar pattern when compared to the other sites (Table 5, Fig. 3). In the fall of 1978, standing crop of this segment was low (12 g/m^2 in December) and was only 6 g/m^2 in March, 1979. Standing crop subsequently increased in the spring and early summer of 1979. However, instead of declining in the fall period, standing crop increased and in December 1979, there was 130 g/m^2 of roots and rhizomes. Throughout the winter and spring, these standing crop figures remained high and were higher than the year previous. Standing crop of reproductive shoots varied between 1979 and 1980. Their weight accounted for 7% in 1979 to 15 to 27% in 1980 of total weight and 7% and 10 to 15% of total plant biomass (Table 5, Figs. 2 and 12).

Vegetative shoot density was lowest both years (1978-1979) in September when density of shoots rapidly began to increase (Table 5, Fig. 4). In 1979, there appeared to be a relatively similar number of shoots between April and August, a decline in September, and then another increase beginning in October. Shoot density in 1980 was maximal in March with 2961 $shoot/m^2$ and then a steady decline after this. The number of shoots in 1980 were much higher than those found the previous year between January and April.

Shoot length was longest in the June-July period and smallest the preceding March (Table 5, Fig. 5). The frequency histograms for this site showed similar patterns to the other sites with new shoot formation in the fall, a large percentage of shoots in the smallest size classes in the spring, rapid elongation of these shoots shifting the shape of the histogram towards larger size classes and then defoliation of the longest leaves and reduction in number of shoots in the late summer (Fig. 13).

One interesting aspect of this particular area was a distinct difference in the number of reproductive shoots in several areas of the bed. This bed, as discussed in the study site section, had a well formed protective sand bar that occurred between the bed and the main stem of the Bay. This sand bar has been shown to be encroaching on the outer edges of the bed (Orth et al., 1979) and it was along this edge that we observed a large number of reproductive shoots in 1979. A comparison of growth parameters of *Zostera marina* in the main *Zostera* bed and near the interface of the sand bar and *Zostera* bed revealed three times more vegetative shoots per m^2 in the *Zostera* bed but almost seven times more reproductive shoots near the interface (Table 6). Twenty six percent of the total number of shoots per m^2 were reproductive at this interface compared to 2% in the *Zostera* bed.

TABLE 5. MONTHLY GROWTH PARAMETERS FOR ZOSTERA MARINA AT THE ZOSTERA STATION AT VAUCLUSE SHORES.

Date	Number of shoots/ m^2 ± 1 SD	Mean Length (cm) ± 1 SD	Shoot Standing Crop (g/m^2) ± 1 SD	Root and Rhizome Standing Crop (g/m^2) ± 1 SD	Total Biomass (g/m^2) ± 1 SD
Sept. 29, 1978	648 ± 352	21.2 ± 0.5	28 ± 7	18 ± 9	46 ± 16
Oct. 26, 1978	838 ± 126	18.9 ± 1.4	44 ± 12	16 ± 4	60 ± 15
Dec. 6, 1978	1855 ± 347	15.3 ± 0.9	38 ± 15	12 ± 4	50 ± 19
March 6, 1979	1228 ± 335	10.4 ± 0.3	12 ± 3	6 ± 2	18 ± 5
April 10, 1979	1600 ± 254	11.4 ± 0.5	27 ± 5	20 ± 5	47 ± 10
April 30, 1979	1538 ± 182	21.7 ± 1.8	51 ± 4	36 ± 6	91 ± 9
	35 ± 29*	29.7 ± 5.9*	4 ± 5*		
May 23, 1979	1445 ± 177	30.5 ± 4.5	95 ± 10	60 ± 5	161 ± 9
	28 ± 17*	40.8 ± 3.4*	7 ± 5*		
June 25, 1979	930 ± 233	36.3 ± 8.5	133 ± 42	70 ± 24	203 ± 58
July 23, 1979	1636 ± 410	24.2 ± 4.6	161 ± 33	90 ± 48	251 ± 41
Aug. 22, 1979	1485 ± 115	22.3 ± 1.7	155 ± 30	85 ± 24	240 ± 24
Sept. 26, 1979	945 ± 300	24.5 ± 2.6	67 ± 9	61 ± 18	127 ± 24
Oct. 25, 1979	1673 ± 348	22.9 ± 1.5	106 ± 18	103 ± 36	210 ± 50
Dec. 11, 1979	2621 ± 273	13.5 ± 0.8	124 ± 30	130 ± 42	254 ± 61
Jan. 17, 1980	2748 ± 433	12.7 ± 1.4	64 ± 6	94 ± 30	158 ± 33
Feb. 14, 1980	2282 ± 624	13.5 ± 0.8	73 ± 15	103 ± 39	176 ± 48
March 12, 1980	2961 ± 642	11.8 ± 0.3	54 ± 6	109 ± 30	164 ± 36
April 30, 1980	1979 ± 215	24.9 ± 0.7	103 ± 6	121 ± 27	265 ± 18
	333 ± 79*	33.6 ± 4.9*	39 ± 9*		
May 28, 1980	1433 ± 430	39.5 ± 0.9	194 ± 46	106 ± 61	334 ± 134
	212 ± 133*	29.9 ± 3.1*	33 ± 33*		
July 1, 1980	1100 ± 173	40.0 ± 1.5	230 ± 21	70 ± 12	300 ± 18
August 7, 1980	1727 ± 658	24.7 ± 3.4	215 ± 33	94 ± 15	306 ± 48

*represents measurements for reproductive shoots during period of sexual reproduction.

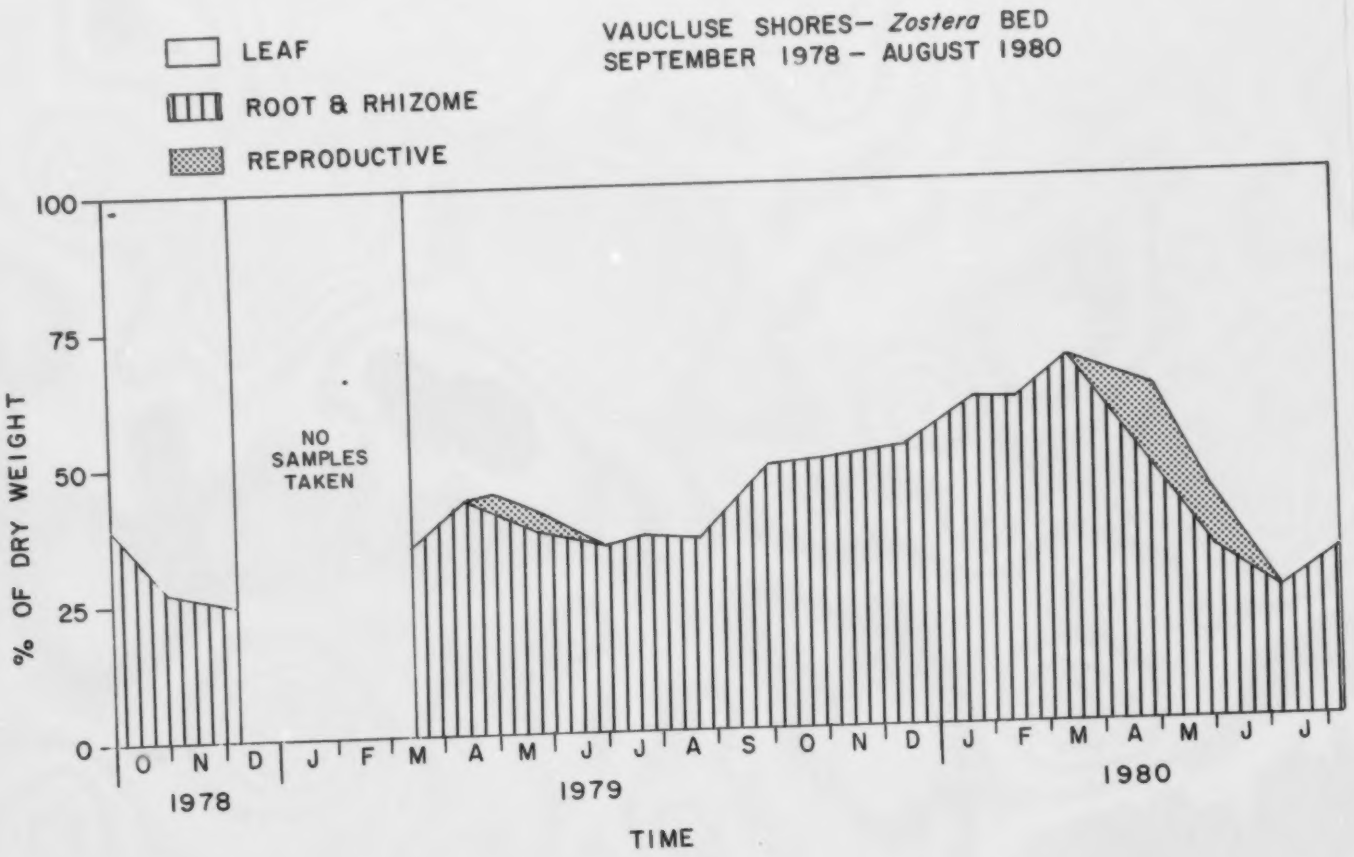


Fig. 12. Partitioning of total biomass of Zostera marina into leaf, root and rhizomes and reproductive fractions based on percent dry weight for the Vaucluse Shores, Zostera bed site. (see Table 5 for raw data).

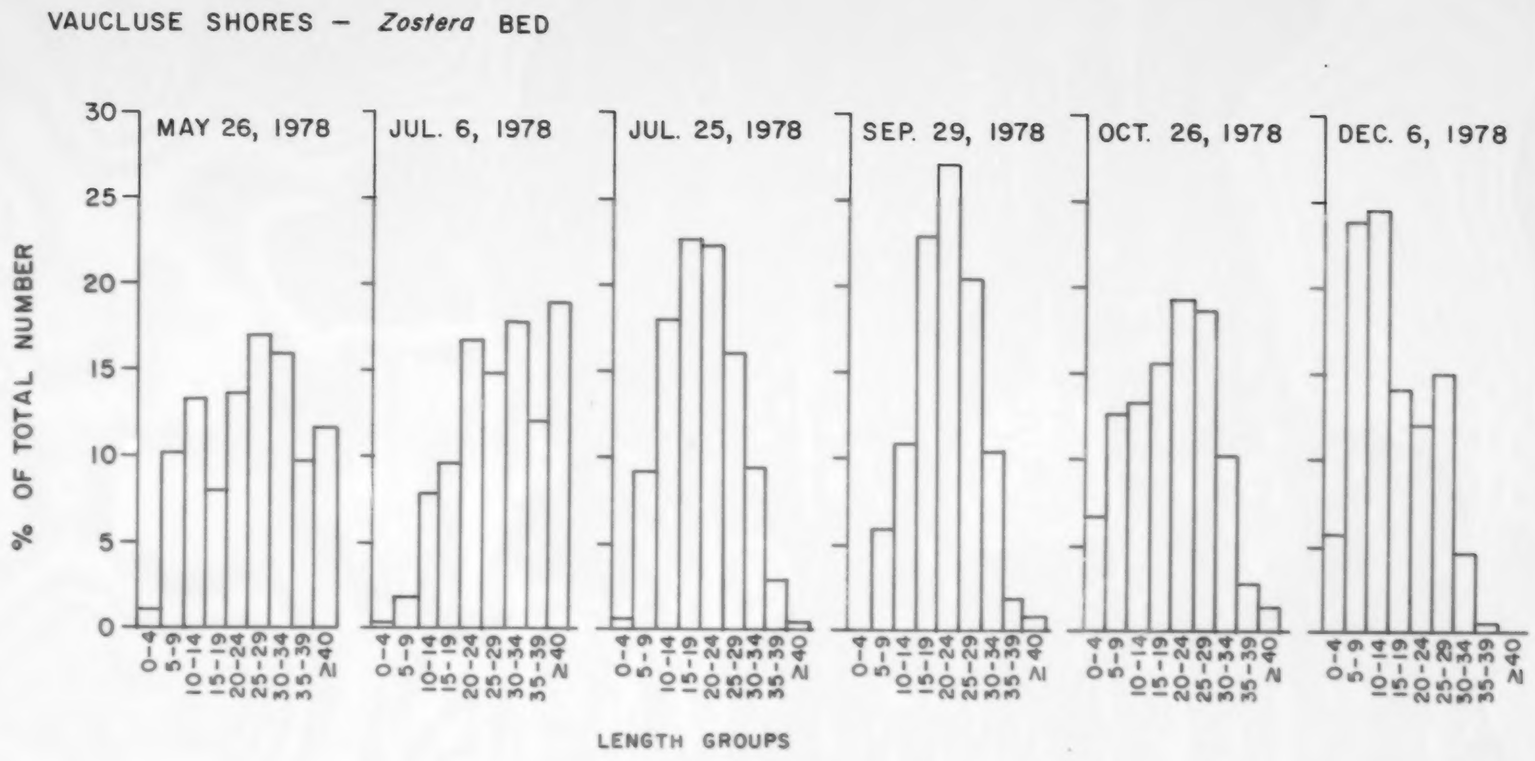
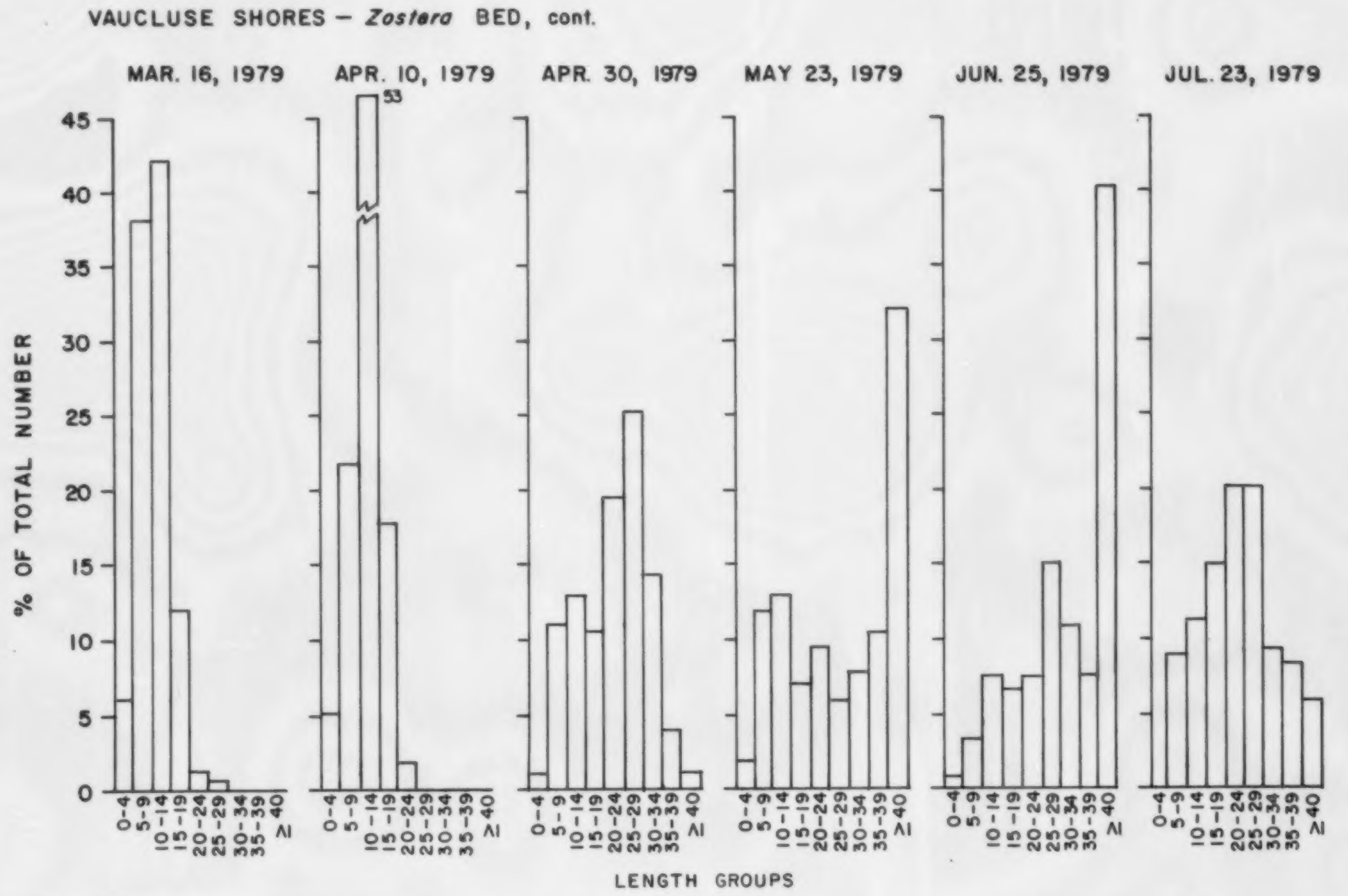


Fig. 13. Frequency histograms of the percent of vegetative shoots in the different size class categories for all sampling periods for *Zostera marina* at the Vaucluse Shores *Zostera* bed site.



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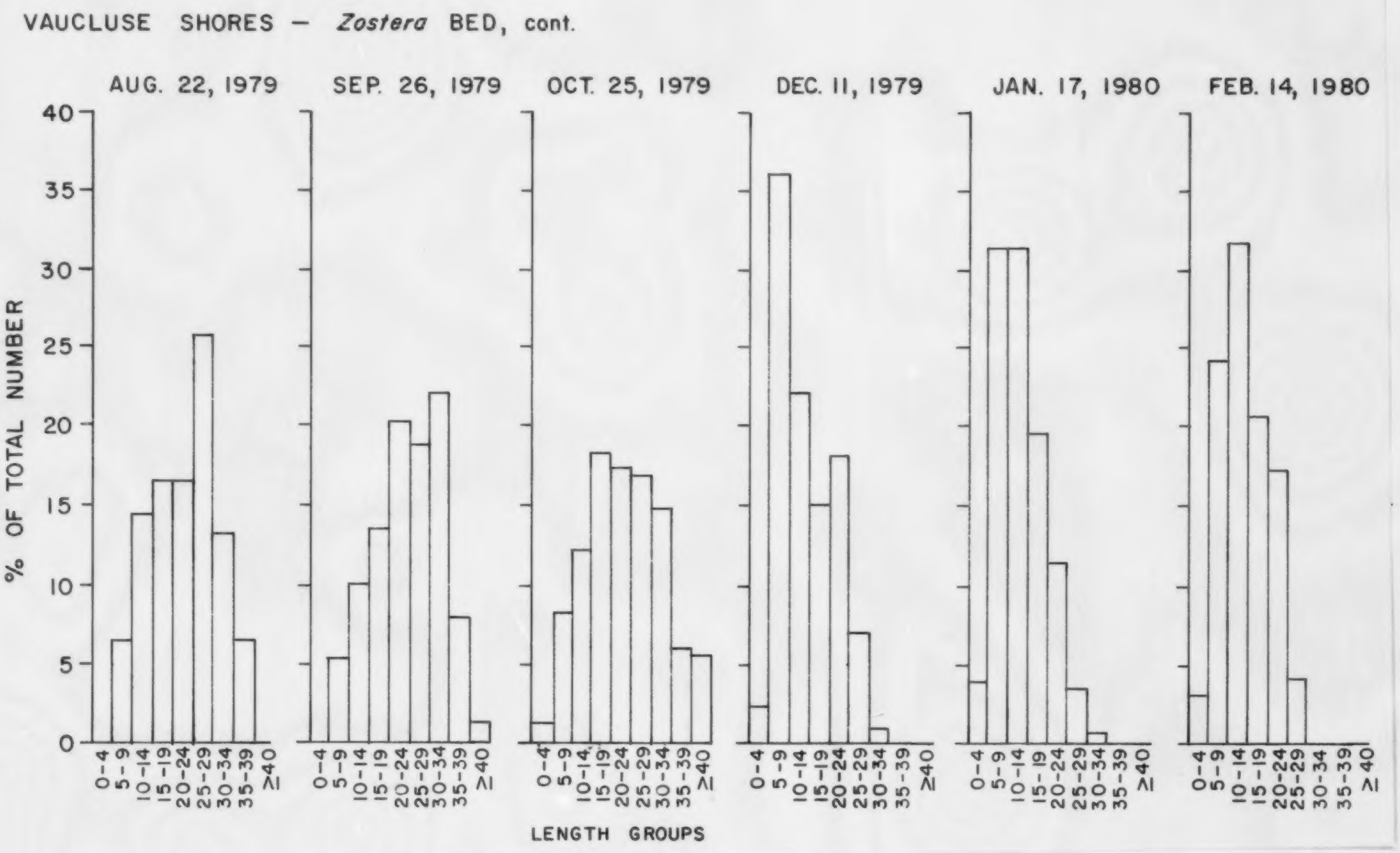

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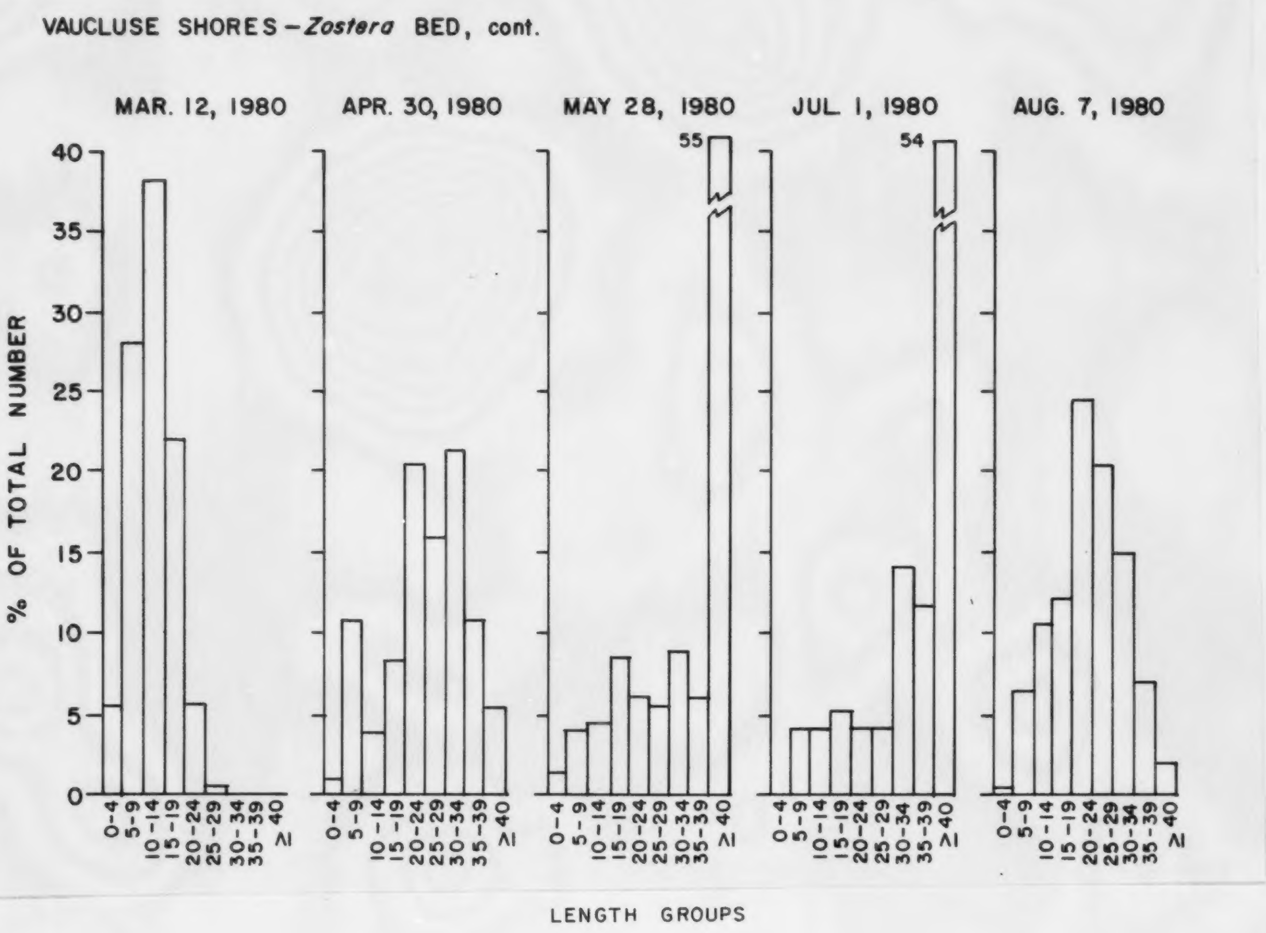

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TABLE 6. COMPARISON OF ZOSTERA VEGETATIVE AND REPRODUCTIVE DATA AT TWO DIFFERENT SITES IN THE GRASS BED AT VAUCLUSE SHORES. FOUR SAMPLES WERE COLLECTED AT EACH SITE WITH A 0.1 m^2 RING. ALL DATA ARE EXTRAPOLATED TO A PER m^2 BASIS (± 1 STANDARD DEVIATION).

	Zostera Bed 5/23/79	6/7/79 Interface Between Inside Edge of Sandbar and *Zostera* Bed
No. of Vegetative Shoots / m^2	1445 ± 177	530 ± 178
Mean Length (cm)	30.5 ± 4.5	38.4 ± 6.7
Shoot Standing Crop (g/m^2)	95 ± 10	127 ± 53
Root and Rhizome Standing Crop (g/m^2)	60 ± 5	34 ± 13
Total Biomass (g/m^2)	155 ± 15	161 ± 66
No. of Reproductive Shoots/ m^2	28 ± 17	182 ± 59
Mean Length (cm)	40.8 ± 3.4	55.8 ± 5.3
Total Biomass (g/m^2)	7 ± 5	90 ± 30
Reproductive Shoots/ Total No. of Shoots	2%	26%

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Vaucluse Shore Mixed Bed

Peak shoot standing crop at this area also occurred during the June-July period for both years (Table 7, Fig. 2). Lowest standing crop during the limited sampling period occurred in May 1979 (37 g/m^2) while during the one winter period that the sampling included, standing crop was lowest in January 1980 (52 g/m^2). Vegetative shoot standing crop averaged 54% (range 34 to 65%) of total plant biomass (Fig. 14). Root and rhizome standing crop was highest in July 1979 but in 1980 there was more root and rhizome standing crop in February (130 g/m^2) than in July (103 g/m^2) (Table 7, Fig. 3). Lowest root and rhizome standing crop occurred in January, 1980 (52 g/m^2) but there also was a low amount in the first sampling period in May 1979 (30 g/m^2). Reproductive shoot standing crop was 11 to 19% of total shoot weight but 8 to 10% of total plant biomass in 1980 (Table 7, Figs. 5, 2 & 14).

Shoot density was highest in July 1979 but lowest number of shoots occurred in June, one month before the July sampling (Table 7, Fig. 4). The lowest number of shoots in the fall occurred in September (1091/m^2). Highest density in 1980 occurred in February (3282/m^2) and remained high through July. Shoot length was highest in May of both years and lowest in the February-March period, 1980 (Table 7, Fig. 5). However, mean shoot length at this site is lower than all other sites except for the Browns Bay site. The frequency histograms for shoots at this site (Fig. 15) are not as clear cut as at the other sites but do show some of the same patterns as discussed for the other sites. The production of new shoots in the late fall and early spring account for the large percentage of shoots in the smaller size classes and subsequent spring growth of these shoots results in greater percentages in the larger size classes.

Vaucluse Shores - *Ruppia*

Widgeon grass, *Ruppia maritima*, was present in a large area of the Vaucluse Shores site. *R. maritima* was present at the mixed bed site, co-occurring with *Zostera marina*, as well as at an inshore site, the *Ruppia* station, where *R. maritima* predominated.

Growth parameters for *Ruppia maritima* at the mixed site are presented in Table 8 and the *Ruppia* site in Table 9. At the mixed station, density of shoots was greatest from June through July in 1979 and 1980 and fewest in the winter months. Trends for shoot standing crop were similar to shoot density. There was not much difference in the mean length of *R. maritima* over the sampling period (range of 4.4 to 9.0 cm) with the shortest length found in March 1979 and the longest in May 1979.

Compared to the mixed bed, *Ruppia maritima* at the *Ruppia* stations was much more dense (Table 9). There were no clearly identified trends for the growth parameters as measured here. *R. maritima* is found in large patches throughout this one and the variation in these patches from where samples were taken may have masked any significant trends. Direct observation indicated that *R. maritima* had a distinct seasonal cycle with a reproductive period that occurred during the late summer period (July-August). During the

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TABLE 7. MONTHLY GROWTH PARAMETERS FOR ZOSTERA MARINA AT THE MIXED STATION AT VAUCLUSE SHORES.

Date	Number of shoots/ m^2 ± 1 SD	Mean Length (cm) ± 1 SD	Shoot Standing Crop (g/m^2) ± 1 SD	Root and Rhizome Standing Crop (g/m^2) ± 1 SD	Total Biomass (g/m^2) ± 1 SD
April 30, 1979	1445 ± 693	17.8 ± 1.8	37 ± 8	20 ± 9	57 ± 16
May 23, 1979	1580 ± 295	22.8 ± 3.7	70 ± 13	48 ± 3	123 ± 13
	50 ± 38*	22.9 ± 3.2*	4 ± 3*		
June 25, 1979	1061 ± 318	16.7 ± 1.8	82 ± 27	64 ± 27	146 ± 55
July 23, 1979	1864 ± 363	18.0 ± 1.8	138 ± 32	112 ± 56	250 ± 79
Aug. 22, 1979	1494 ± 400	15.6 ± 2.1	97 ± 30	73 ± 42	170 ± 70
Sept. 26, 1979	1091 ± 276	19.3 ± 2.6	70 ± 18	67 ± 21	137 ± 36
Oct. 25, 1979	1439 ± 376	20.9 ± 1.7	85 ± 24	64 ± 24	148 ± 30
Dec. 11, 1979	2252 ± 467	12.0 ± 0.8	70 ± 21	106 ± 30	164 ± 52
Jan. 17, 1980	2394 ± 182	11.5 ± 1.4	52 ± 12	52 ± 9	100 ± 21
Feb. 14, 1980	3282 ± 730	10.0 ± 0.4	64 ± 9	130 ± 70	191 ± 79
March 12, 1980	2264 ± 464	10.5 ± 0.7	58 ± 18	94 ± 24	148 ± 9
April 30, 1980	2888 ± 955	14.2 ± 1.5	76 ± 18	82 ± 3	175 ± 16
	212 ± 6*	20.0 ± 3.2*	18 ± 3*		
May 28, 1980	2130 ± 18	20.8 ± 2.0	142 ± 27	79 ± 9	239 ± 39
	173 ± 97*	19.9 ± 2.3*	18 ± 12*		
July 1, 1980	1818 ± 709	17.3 ± 1.8	161 ± 39	103 ± 29	264 ± 58
August 7, 1980	1294 ± 282	16.4 ± 1.8	94 ± 24	97 ± 58	194 ± 76

*represents measurements for reproductive shoots during period of sexual reproduction.

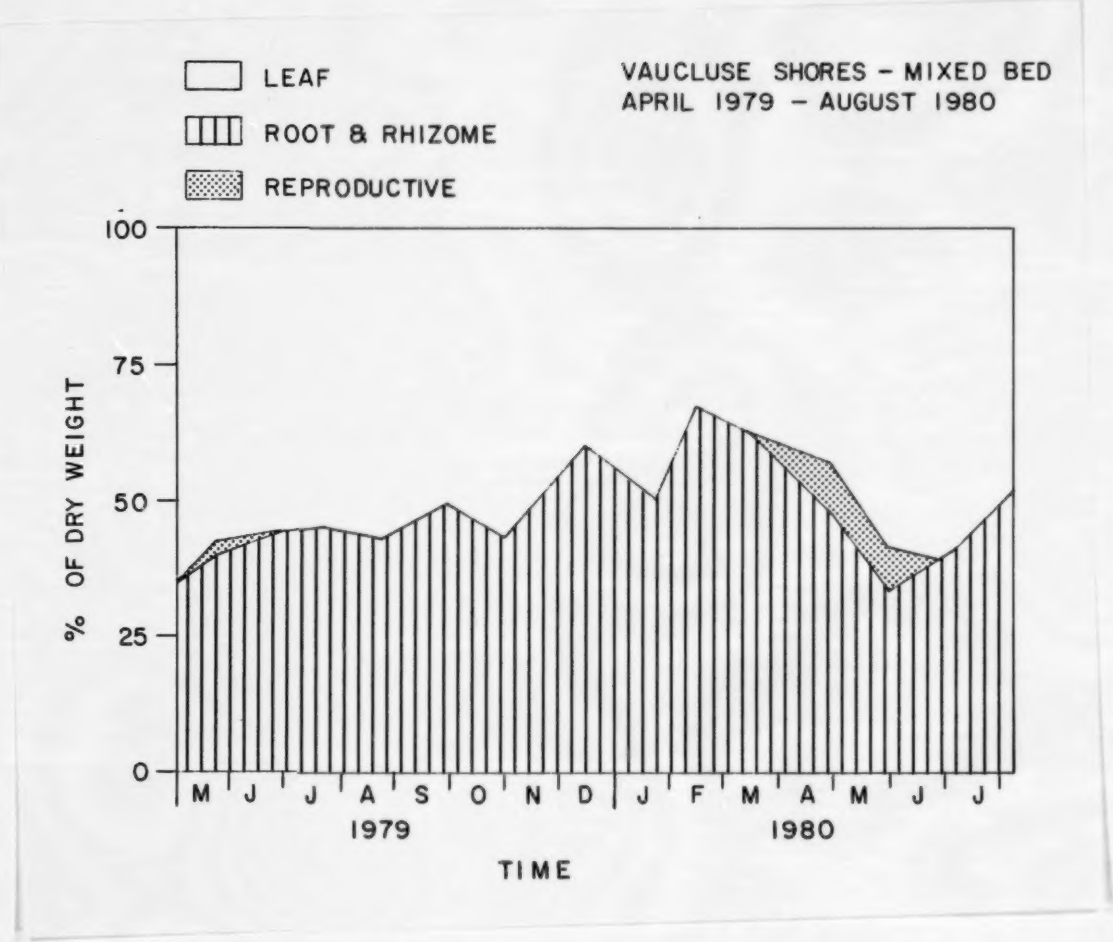

Fig. 14. Partitioning of total biomass of Zostera marina into leaf, root and rhizome and reproductive fractions based on percent dry weight for the Z. marina at the Vaucluse Shores Mixed Bed site (see Table 6 for raw data).

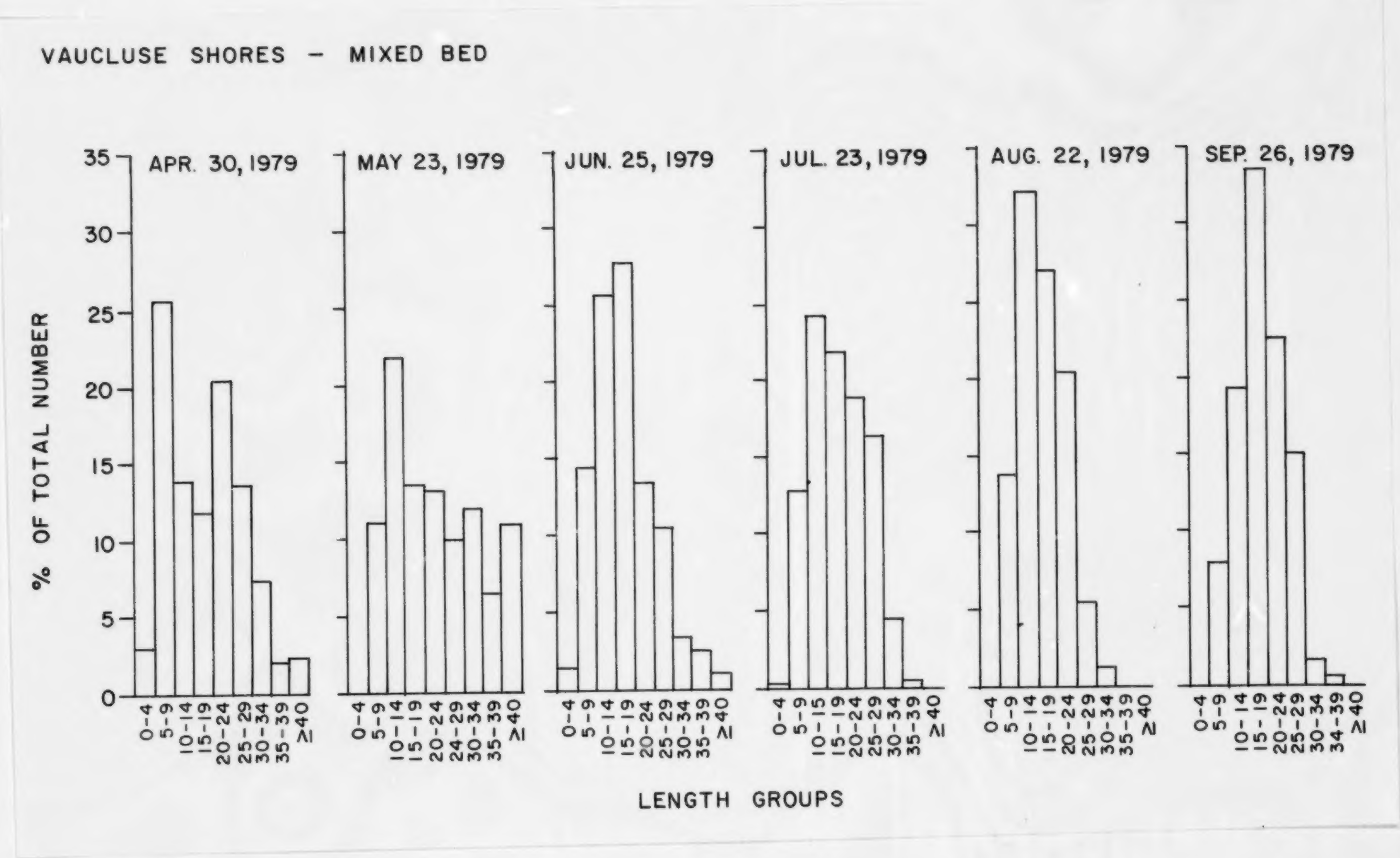

Fig. 15. Frequency histograms of the percent of vegetative shoots in the different size class categories for all sampling periods for *Zostera marina* at the Vaucluse Shores Mixed bed site.

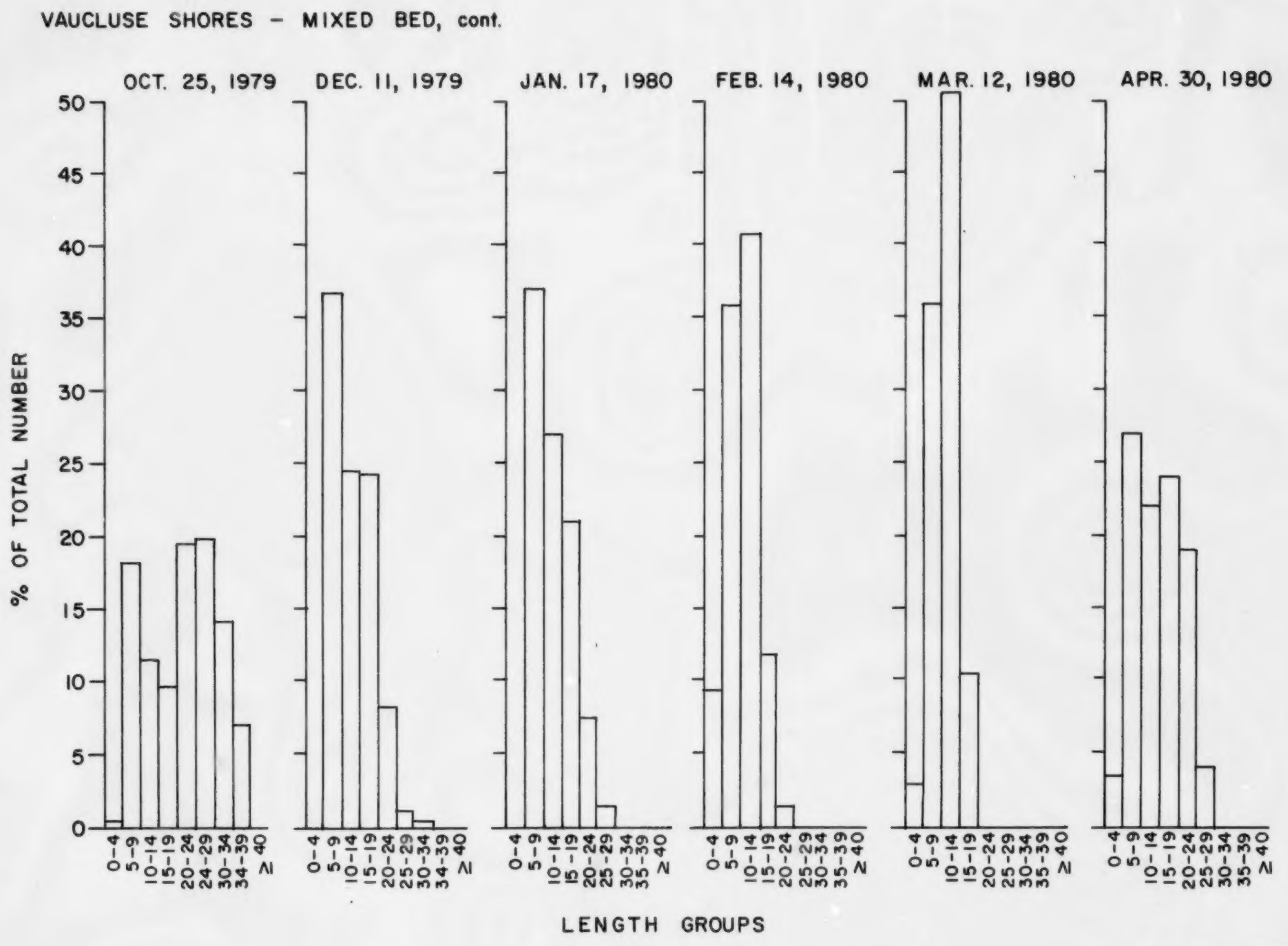

Fig. 15 (continued)

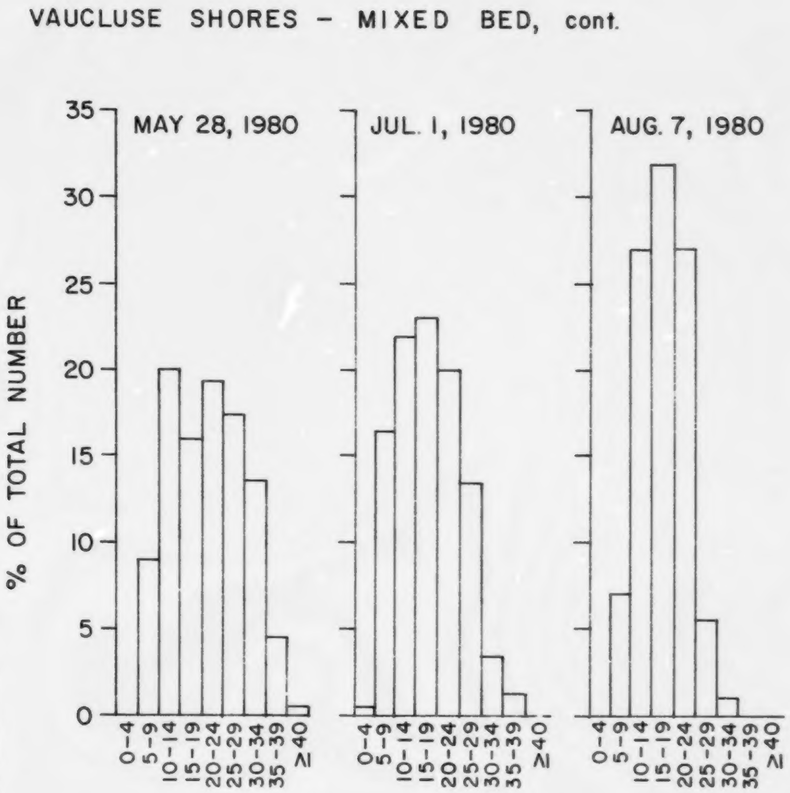

Fig. 15 (continued)

TABLE 8. MONTHLY GROWTH PARAMETERS FOR RUPPIA MARITIMA AT THE MIXED STATION AT VAUCLUSE SHORES.

Date	Number of shoots/ m^2 ± 1 SD	Mean Length (cm) ± 1 SD	Shoots Standing Crop (g/m^2) ± 1 SD	Roots and Rhizomes Standing Crop (g/m^2) ± 1 SD	Total Biomass (g/m^2) ± 1 SD
May 1, 1979	6702 ± 2848	7.5 ± 1.2	17 ± 5	17 ± 4	34 ± 9
May 23, 1979	—*	9.0 ± 0.7			43 ± 6
June 25, 1979	13,145 ± 5245	7.9 ± 1.0	30 ± 12	21 ± 9	51 ± 20
July 23, 1979	12,717 ± 6545	7.5 ± 1.0	45 ± 31	27 ± 21	72 ± 52
Aug. 22, 1979	11,721 ± 6061	6.2 ± 0.7	39 ± 12	24 ± 9	63 ± 23
Sept. 26, 1979	5782 ± 2297	6.9 ± 0.5	18 ± 6	15 ± 6	33 ± 11
Oct. 25, 1979	6567 ± 2845	7.6 ± 1.6	18 ± 9	12 ± 6	29 ± 12
Dec. 11, 1979	5324 ± 3145	6.9 ± 0.7	15 ± 9	21 ± 15	39 ± 24
Jan. 17, 1980	6273 ± 4094	7.2 ± 1.1	21 ± 3	24 ± 3	47 ± 2
Feb. 14, 1980	4779 ± 4130	5.5 ± 0.3	12 ± 12	15 ± 15	27 ± 27
March 12, 1980	3615 ± 448	4.4 ± 0.4	6 ± 0	6 ± 6	12 ± 6
April 30, 1980	5191 ± 1594	5.7 ± 0.2	12 ± 6	12 ± 6	21 ± 12
May 23, 1980	9252 ± 3179	5.9 ± 0.6	21 ± 6	18 ± 12	39 ± 18
July 1, 1980	10,052 ± 4612	7.1 ± 1.7	30 ± 12	18 ± 9	48 ± 21
Aug. 7, 1980	15,052 ± 2273	8.9 ± 1.1	58 ± 18	33 ± 9	91 ± 24

*No. of shoots and individual biomass data not taken here

TABLE 9. MONTHLY GROWTH PARAMETERS FOR RUPPIA MARITIMA AT THE RUPPIA STATION IN VAUCLUSE SHORES.

Date	Number of Shoots/ m^2 ± 1 SD	Mean Length (cm) ± 1 SD	Shoot Standing Crop (g/m^2)	Root and Rhizome Standing Crop (g/m^2) ± 1 SD	Total Biomass (g/m^2) ± 1 SD
May 2, 1979	10,286 ± 4514	4.7 ± 0.2	9 ± 8	29 ± 14	38 ± 23
June 25, 1979	14,727 ± 3046	6.8 ± 0.6	30 ± 9	45 ± 15	75 ± 22
July 23, 1979	12,086 ± 4378	6.9 ± 0.4	29 ± 12	30 ± 15	59 ± 26
Aug. 22, 1979	17,166 ± 9715	7.2 ± 0.7	30 ± 12	33 ± 9	63 ± 18
Sept. 26, 1979	15,855 ± 5367	7.5 ± 0.7	70 ± 24	76 ± 27	146 ± 51
Oct. 25, 1979	22,824 ± 4961	9.0 ± 0.6	85 ± 15	79 ± 9	164 ± 19
Dec. 11, 1979	19,630 ± 5158	6.8 ± 1.0	61 ± 24	91 ± 12	148 ± 33
Jan. 17, 1980	16,606 ± 1585	6.8 ± 0.8	58 ± 3	106 ± 15	165 ± 15
Feb. 14, 1980	13,524 ± 2470	5.3 ± 1.3	46 ± 18	67 ± 33	112 ± 24
March 12, 1980	12,991 ± 4139	6.7 ± 0.6	48 ± 18	48 ± 6	97 ± 18
April 30, 1980	25,373 ± 2673	5.1 ± 0.5	52 ± 21	88 ± 36	139 ± 21
May 28, 1980	11,052 ± 2100	6.0 ± 0.8	30 ± 12	46 ± 12	73 ± 24
July 1, 1980	18,870 ± 3673	6.9 ± 1.0	70 ± 12	76 ± 15	146 ± 24
Aug. 7, 1980	30,494 ± 5024	7.5 ± 0.5	136 ± 48	100 ± 15	236 ± 45

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winter months, growth is reduced as evidenced by presence of only very small shoots.

Temperature, Salinity, Sediments

Continuous temperature recordings taken at the Virginia Institute of Marine Science provided more detailed information on temperature patterns during the course of this study (Fig. 16). Although temperature in the shallows may fluctuate during the day, temperatures taken at the sampling sites during routine sampling, as well as from other sub-projects (e.g. the seed germination experiments), revealed very similar trends as that provided by the permanent recording equipment.

Minimal water temperatures occurred in January or February in all three years with lowest recorded temperatures approaching 1°C. Temperatures in the shallows where the grasses occurred probably were close to 0°C or less because of the fact that these areas had ice coverage during the winter period.

Maximal summer temperatures were reached in July or August with temperatures reaching 29°C in each year. Temperatures between the summer maxima and the winter minimum were also similar for 1978, 1979 and 1980.

Continuous salinity measurements were not available from VIMS. However, salinity samples taken routinely during the study period indicated salinities at all three sites for the biomass sampling to be quite similar. Salinities were always lowest in the spring and highest in the late summer or fall. Salinity range in 1979 was 12.4 to 19.0 $^{o}/oo$, while in 1980 it was 15.8 to 24.5 $^{o}/oo$. The latter half of 1980 was extremely dry, with little runoff from land, accounting for the higher salinities recorded that year.

Sediments at the five sites consisted primarily of sand with lesser percentages of silt and clay (Table 10). The Guinea Marsh inshore site, which is more protected as well as being fronted by the large, expansive grass flats has more silt and clay than the other sites. The quiet water conditions here would allow finer sediments to accumulate. Median grain size ranged from 2.4 ϕ at the Browns Bay site to 3.1 ϕ at the Guinea Marsh inshore area

DISCUSSION

Despite some differences that existed among the sampling sites for the measured parameters, several trends were evident from the data. One very interesting aspect was that there were large differences between years for both maximum and minimum values (Tables 11 and 12) of parameters such as shoot standing crop, shoot density and number of reproductive shoots. The standing crop of vegetative shoots was always highest in the June-July period at all sites while minimal values for standing crop occurred during the fall or winter months in both years. However, the standing crop of *Zostera marina* during the June-July period in 1980 was higher compared to 1979 at all sites. The fact that all sites showed this trend suggests that possibly the grass

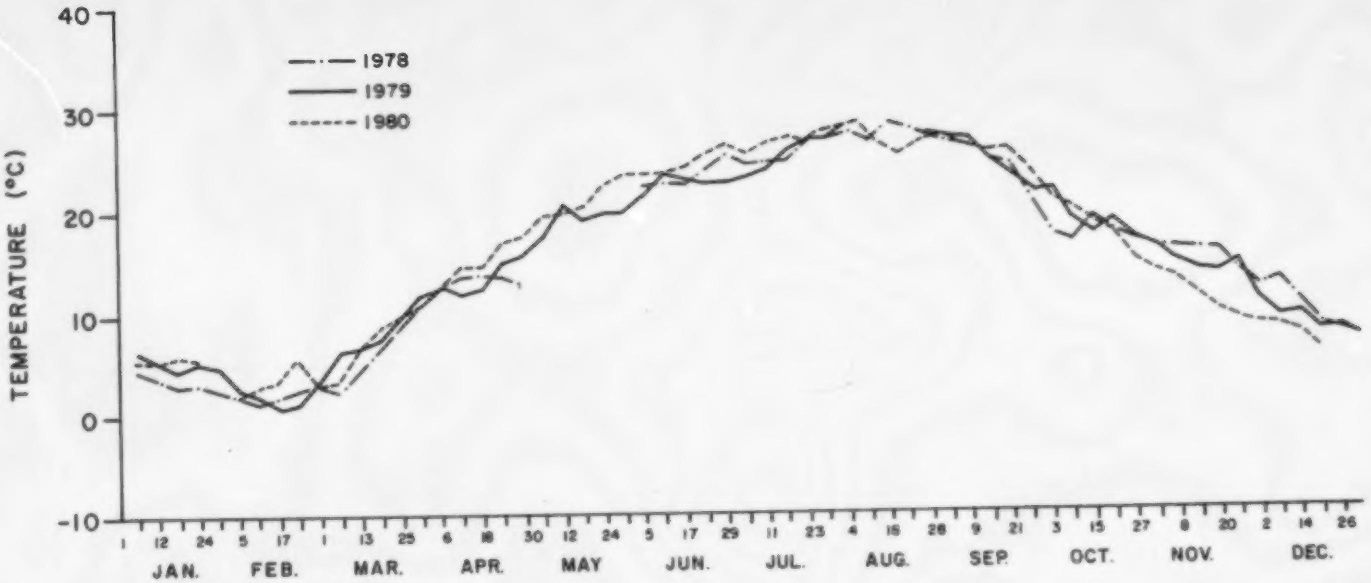

Fig. 16. Water temperature recorded from the Virginia Institute of Marine Science for 1978, 1979 and 1980 (points represent 6 day averages of continuous daily records).

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TABLE 10. PERCENT SAND AND SILT AND CLAY IN SEDIMENTS COLLECTED FROM THE STUDY SITES AND MEDIAN GRAIN SIZE (PHI UNITS, ϕ, where $\phi = -\log_2$ mm).

	Sand	Silt & Clay	Median (Md_ϕ)
Browns Bay			
June, 1980	88.5	11.5	2.4
Nov., 1980	85.0	15.0	2.5
Guinea Marsh			
Offshore	86.4	13.6	2.6
Inshore	77.3	22.7	3.1
Vaucluse Shores			
Zostera	91.5	8.5	2.8
Mixed	92.2	7.8	2.9

TABLE 11. MAXIMUM AND MINIMUM VALUES FOR SHOOT AND ROOT-RHIZOME STANDING CROP FOR ALL SITES (MONTHS IN PARENTHESIS ARE FOR WHEN THE VALUE WAS RECORDED) SOME SITES WERE NOT SAMPLED FOR THE ENTIRE YEAR (DATA FROM TABLES 2, 3, 4, 5 and 7).

	Shoot Standing Crop (g/m^2)		Root-Rhizome Standing Crop (g/m^2)	
	Max.	Min.	Max.	Min.
Browns Bay				
1978		23(Oct.)		6(Oct.)
1979	161(July)	9(Sept) 11(March)	155(July)	15(Sept) 8(March)
1980	173(June)	48(March)	206(June)	48(March)
Guinea Marsh Offshore				
1978	158(Aug.)	57(Oct.)	105(June)	10(Oct.)
1979	336(June)	70(Nov.) 34(March)	130(June,July)	42(Nov.) 10(March)
1980	397(July)	33(March)	155(June)	88(Feb.)
Guinea Marsh Inshore				
1979	291(June)	9(Oct.)	61(June)	3(Nov.)
1980	412(July)	2(Jan.)	121(July)	1(Jan.)
Vaucluse Shores *Zostera*				
1978		28(Sept.)		12(Dec.)
1979	161(July)	12(March)	130(Dec.)	61(Sept.) 6 (March)
1980	230(July)	54(March)	121(April)	103(Feb.)
Vaucluse Shores Mixed				
1979	138(July)	37(May)	112(July)	20(May)
1980	161(July)	52(Jan.)	130(Feb.)	52(Jan.)

TABLE 12. MAXIMUM AND MINIMUM VALUES FOR VEGETATIVE SHOOT DENSITY AND SHOOT LENGTH AND MAXIMUM NUMBER OF REPRODUCTIVE SHOOTS PLUS ITS PERCENTAGE OF TOTAL SHOOTS FOR ALL SITES (MONTHS IN PARENTHESIS ARE FOR WHEN THAT VALUE WAS RECORDED). SOME SITES WERE NOT SAMPLES FOR THE ENTIRE YEAR (DATA FROM TABLES 2, 3, 4, 5, and 7).

	Shoot Density (No./m^2)		Shoot Length (cm)		Reproductive Shoots (No./m^2)
	Max.	Min.	Max.	Min.	
Browns Bay					
1978		788(Oct.)			
1979	2576(July)	591(Sept.)	19.6(June)	8.3(March)	192(11%)
1980	2918(March)		15.1(July)	8.0(March)	424(20%)
Guinea Marsh Offshore					
1978	1578(Aug.)	695(Oct.)	22.8(Aug.)		
1979	1970(Oct.)	900(Sept.)	40.2(June)	12.4(March)	365(17%)
1980	3030(Feb.)		37.5(July)	10.8(March)	303(11%)
Guinea Marsh Inshore					
1979	1438(April)	206(Aug.)	59.7(June)		368(21.5%)
1980	2597(June)		25.5(July)	5.9(March)	82(3.4%)
Vaucluse Shores *Zostera*					
1978		648(Sept.)			
1979	1636(July)	945(Sept.)	36.3(June)	10.4(March)	35(2%)
1980	2961(March)		40.0(July)	11.8(March)	333(14%)
Vaucluse Shores Mixed					
1979	1864(July)	1861(June)	22.8(May)		50(3%)
1980	3283(Feb.)	1294(Aug.)	20.8(May)	10.0(Feb.)	212(7%)

beds may be responding to some external environmental control for biomass production (e.g. temperature) or even a biological control mechanism [e.g. waterfowl interactions (Jupp and Spence, 1977)] that affects all grass beds, and which can vary from year to year.

Root-rhizome standing crop followed similar trends as the shoot standing crop except for some variation at the Vaucluse Shore sites.

The pattern of growth as expressed in shoot denisty was different than the shoot standing crop. Lowest density of shoots occurred in the late summer to early fall period, while highest density occurred in the spring and early summer period. Appearance and growth of new shoots were not observed to occur after mid-August which coincides with the defoliation period when older leaves and shoots slough off after water temperatures peaked between 25 and 30°C. New shoots were first observed after the summer dieback around the end of September and early October as evidenced by the appearance of new, small (<5cm) shoots. New shoots appeared to be constantly produced throughout the winter and spring as confirmed by visual examination of the biomass samples during processing and the plots of the size frequency histograms. The large production of new shoots in the fall and winter resulted in this period having very high shoot densities compared with the early summer period prior to the defoliation of the older leaves. The reduction in shoot density from the spring to the early summer period may be a result of a self shading mechanism or temperature stress. Although data are not available, it is possible that as leaves rapidly elongate in the spring, sufficient light may not reach the sediment surface where the new shoots would be found, especially in very dense beds, to allow these new shoots to grow.

The mean length of the shoots showed a distinct trend for all sites. Leaf elongation began around March and continued through the June-July period where the mean length was always longest (*Zostera marina* at the Vaucluse Shores mixed site reached peak length in May but this may be a result of temperatures rising faster in this shallower area compared to the deeper *Zostera* site, thus causing *Z. marina* to grow faster here). Leaf length decreased from mid-summer to March as a result of loss of longer older leaves and shoots in the late summer along with the increased production of new, smaller shoots in the late fall and winter period. This was evident in the frequency diagrams of the different size class categories which always showed a large percentage of small shoots in the March period.

The contribution of seedlings and subsequent seedling growth in *Zostera marina* beds can be highly variable because of differential seed recruitment which is dependent upon not only seed production within a particular area but possible seed dispersal from other areas. The number of reproductive shoots in a particular area can be highly variable from year to year. The number of seedlings observed at the Guinea Marsh inshore area was high. This could be the result of either limited dispersal of seeds produced in this area (the site was semi-protected in a small embayment) or a large dispersal of seeds washed into this area from the adjacent area. This large number of seedlings was the cause for this area to revegetate as rapidly as it did (new shoot production from old rhizome stock was small at this site compared to the other sites (Table 4, Fig. 4). Despite the shorter mean length of shoots in 1980 compared to 1979, the large increase in the number of shoots in 1980 over 1979 resulted in the greater shoot biomass in 1980.

Zostera marina in the Chesapeake Bay exhibits distinct seasonal trends in its growth cycle as observed from all the data collected from the different sites. Growth does occur in terms of new shoot production in all months except from mid-July to mid- to late September. Because of high summer temperatures in these shallow areas where *Z. marina* grows (up to 29°C), growth stops with no new shoot production occurring with the plants. In the early fall as temperatures decline to between 20-25°C, new shoot production from the existing vegetative stock begins and continues through the late spring period. Seed germination (see section on seed germination aspects) begins in late October and continues through, at least, April and possibly early May. Growth of seedlings is rapid especially for the period from March to May. Because of new shoot production, shoot density increases in the fall and remains high until June and July. The smaller, new shoots depresses the mean length until March, when, as the temperatures begin to increase above 5-10°C, rapid growth causes mean length and thus shoot biomass to increase. The period of sexual reproduction occurs from approximately mid- to late February until early June, when all seed have been released.

The trends described above for *Zostera marina* in the Bay parallel those described in other studies of Bay *Z. marina* populations (March, 1970, 1973; Orth and Heck, 1980). Data for other *Z. marina* beds on the East Coast of the United States are limited but are available for North Carolina and Long Island Sound. In North Carolina (Dillon, 1971; Penhale, 1977) *Z. marina* growth and defoliation is shifted by about one month before those events that occur in the Bay while in Long Island Sound (Burkholder and Doheny, 1968) growth occurs approximately one month later than in the Bay. Because of the latitudinal separation of all three sites, we suggest that temperature is a very important factor for regulating the growth of *Z. marina* and that the shift in growth of *Z. marina* proceeding northward appears to be directly related to water temperatures, rising earlier in North Carolina and later in Long Island Sound. Although temperature is an important factor, we also agree with Jacobs and Pierson (1981) that irradiance also varies with latitude, and that this may have subtle effects on the phenology. Bachman and Barilotti (1976) concluded that irradiance was important for flowering. However, before conclusions on the ultimate factors that affect growth, further experimentaion is necessary for elucidation of what influence both temperature and irradiance have on the seasonal growth cycle.

Comparison of seasonal trends in standing stock of *Zostera marina* in the Bay and that in Japan indicate close similarity in patterns for shoot density and standing crop of leaves and roots and rhizomes (Mukai et al., 1979, 1980; Aioi, 1980; Aioi et al., 1980). This would be expected because of the similarity in latitude (37° for Chesapake Bay, 35° for study site in Japan) and similarity in water temperature patterns, although water temperatures in the Bay are colder in the winter months (0°C for Bay, 10°C for Japan). However, the Japanese rsearchers felt that insolation was the critical factor rather than temperature although Aiai et al. (1980) suggested temperature may be essential for differentiation of generative organs.

The data presented here adds to the basic knowledge on the biology of *Zostera marina* in the Chesapeake Bay. Despite these contributions, significant questions remained unanswered: What is the relative contribution of temperature and irradiance to the growth cycle? How do sediment nutrients change seasonally and what is their affect on seagrass production? What is the relative contribution of epiphytes, both macro and micro, sediment flora and seagrass to the total productivity of the system? Is *Zostera marina* nutrient limited in the Bay? What are the factors that allow *Z. marina* and *Ruppia maritima* to coexist in the shallow water but not in deeper water? How do annual changes in runoff affect light quality and quantity at different vegetated sites. How do annual changes in irradiance affect vegetative and reproductive growth? What controls maximum standing crop in an area? Does a vegetated area ever become totally senescent so as to result in a total die back in one year as we observed at a Guinea Marsh site? How important is seed recruitment and germination to the ultimate maintenance of the existing bed? These represent some of the significant areas where future research lies.

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CHAPTER 2

ANTHESIS AND SEED PRODUCTION IN *ZOSTERA MARINA* L. (EELGRASS) FROM THE CHESAPEAKE BAY*

by

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and
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ABSTRACT

Anthesis and seed production in Zostera marina were studied in three areas of the Chesapeake Bay from January to June 1980. Spadix primordia with distinguishable anthers and pistils were first observed in February when water temperature was 3°C. Development of the reproductive shoots in the field continued after February as water temperature rose, with the first evidence of pollen release in mid-April (water temperature 14.3°C). Stigmata loss was first observed in samples taken in late April at two of the areas as water temperatures averaged above 16°C. Pollination was complete at all locations by 19 May and anthers were no longer present. Few reproductive shoots were found on 3-5 June and seed release was assumed to be complete by this time (water temperature 25°C). The density of flowering shoots ranged from 11 to 19% of the total number of shoots, producing an estimated 8127 seeds m^{-2}.

Comparison of flowering events with other areas along a latitudinal gradient from North Carolina to Canada indicated that reproductive events occurred earlier in the most southern locations and at successively later dates with increasing latitude.

INTRODUCTION

Anthesis and seed production are two critical stages in the life cycle of seagrasses. Despite the ultimate importance of flowering in seagrasses, few studies have described, in detail, these processes and the factors that initiate it. Most notable for the species Zostera marina L. are those studies by Churchill and Riner (1978), DeCock (1980, 1981a, 1981b, 1981c) and Jacobs and Pierson (1981). DeCock conducted extensive laboratory studies on Z. marina populations collected from the Netherlands and compared this with field plants while Churchill and Riner (1978) have presented a detailed account of anthesis and seed production in North America Z. marina populations. Except for the Churchill and Riner (1978) study, these aspects have been only briefly reported on in a few other papers for North American Z. marina populations (Setchell, 1929; Taylor, 1957; McRoy, 1970; Dillon, 1971; Phillips, 1972; Keddy and Patriquin, 1978).

The objectives of our work were to describe the timing of the events in the flowering process for lower Chesapeake Bay Zostera marina beds and to compare this information with data available for other locations along the east coast of the United States. The nature of flowering of North American populations and those of European counterparts are also compared.

STUDY SITES, MATERIALS AND METHODS

Zostera marina was collected from three locations in the lower Chesapeake Bay in 1980 to ascertain the timing of the flowering events (Fig. 1). Site 1 was located in the Mobjack Bay near Browns Bay. The dominant vegetation at this site is Z. marina although it co-occurs with Ruppia maritima (widgeon grass). The vegetation in this area is found in a 400 m wide bed parallel to the shoreline. There are approximately 41 ha of bottom covered with vegetation in the immediate vicinity of this site (Orth et al., 1979). The sampling location was at a water depth of 0.5 m MLW (mean low water).

Site 2 was located at the mouth of the York River adjacent to the Guinea Marshes in a monospecific stand of Z. marina. This area is an expansive shoal (<1.5 m MLW) which is almost entirely vegetated by Z. marina. There are approximately 309 ha of bottom covered by Z. marina in and adjacent to this site (Orth et al., 1979). Depth of water at the sampling location was approximately 0.75 m (MLW).

Site 3 was located on the western side of the Eastern Shore of Virginia in an area called Vaucluse Shores. This area is dominated by R. maritima in the very shallow water (0.3 m - MLW), Z. marina in the deeper water

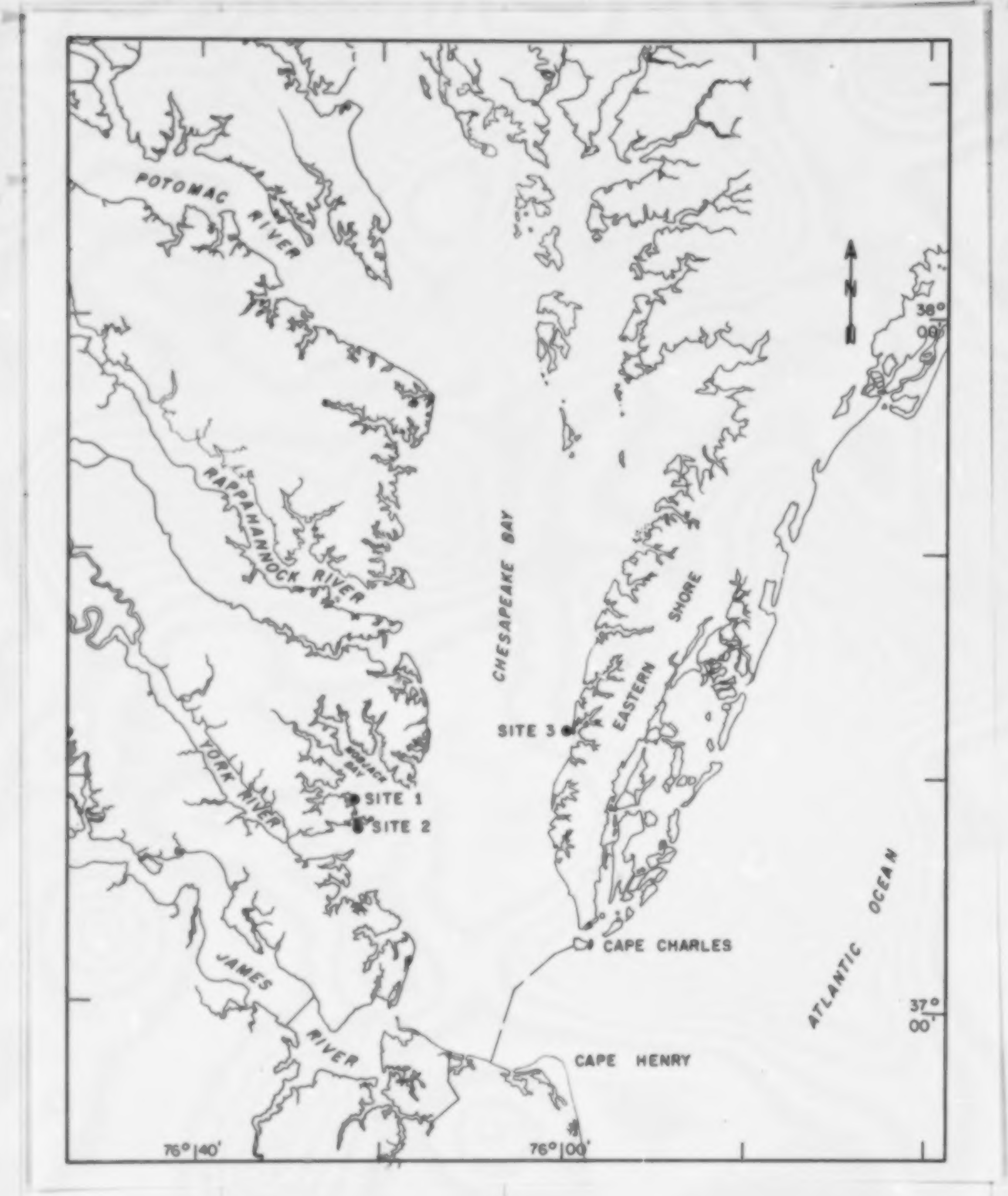
POTOMAC RIVER
RAPPAHANNOCK RIVER
YORK RIVER
JAMES RIVER
MOBJACK BAY
SITE 1
SITE 2
CHESAPEAKE BAY
SITE 3
EASTERN SHORE
CAPE CHARLES
CAPE HENRY
ATLANTIC OCEAN
N
38° 00'
37° 00'
76°40'
76°00'

Fig. 1. Location of the three study sites (1,2,3) in the Chesapeake Bay.

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(>1.0 m MLW) and a mixture of the two grasses at intermediate depths. The vegetation at this site is found between the shoreline and an offshore sandbar, located 500-700 m from shore. There are 211 ha of bottom covered by vegetation in this area (Orth et al., 1979). The sampling location was in the deeper, Z. marina portion where a water depth was approximately 1 m (MLW).

Weekly collections of individual shoots were made beginning 18 January 1980 at each of the sites to ascertain the beginning of the flowering period. Afterwards, replicate samples of 0.033 m^2 were taken using a large, plexiglass corer. The entire core of leaves, roots and rhizomes were placed in a coarse mesh bag, rinsed free of sediments and placed in a bucket of water for later analysis. Water temperatures were either taken at the sites or were obtained from a nearby recording station. Samples were brought to the laboratory and vegetative and reproductive (generative) shoots were separated, counted and recorded. Spadices were dissected from the shoots their position hierarchy noted (terminal rhipidium, rhipidia branches 3,2,1) similar to that defined by DeCock (1981). Selected spadices were preserved in 70% EtOH for further examination. The length of each spadix and number per rhipidium and shoot were determined. Anthers and pistils were counted and size range measurements within each spadix were recorded.

RESULTS

No reproductive shoots were observed at the sampling sites until 14 February 1980. Shoots from the Guinea Marshes (site 2) and Eastern Shore (site 3) contained spadices which ranged from 0.5-3.5 cm long. Anthers were in a primordial stage of development, but could be clearly distinguished as they had obtained their characteristic elongated, elliptical shape (Fig. 2a). The size of the largest ones (4-5 mm) were comparable to anthers collected as late as May. In contrast, pistils were quite immature with many as yet undeveloped. They ranged in size from 0.2-0.8 mm and appeared to be round and bun-like with no differentiation of ovule, style or stigmata. Water temperature readings were 3.0°C for each of the sites. Data on the mean numbers of rhipidium, spadices, pistils, pollens sacs and percent of fertilized embryos for all the sampling dates at the three sites are presented in Table 1. During February and March the reproductive shoots generally contained only one rhipidium with one or two spadices per rhipidium. The ratio of pollen sacs to pistils within the spadices during February was greater than 2:1, reflecting the undeveloped state of the pistils, assuming one pistil will develop with each two pollen sacs in the spadices.

Development of the reproductive shoots in the field continued after Feburary as water temperature increased, with the first evidence of pollen release observed in samples taken on 10 April from site 3. The average water temperature was 14.3°C. By this time, the pistils were fully differentiated into ovule, style and bifurcated stigmata (Fig. 2b) and some were in erection stage as described by DeCock (1980). In these samples, maximum anther length was 6 mm and maximum pistil length was 5 mm. Among the samples taken during March and early April the ratio of pollen sacs to pistils was approximately

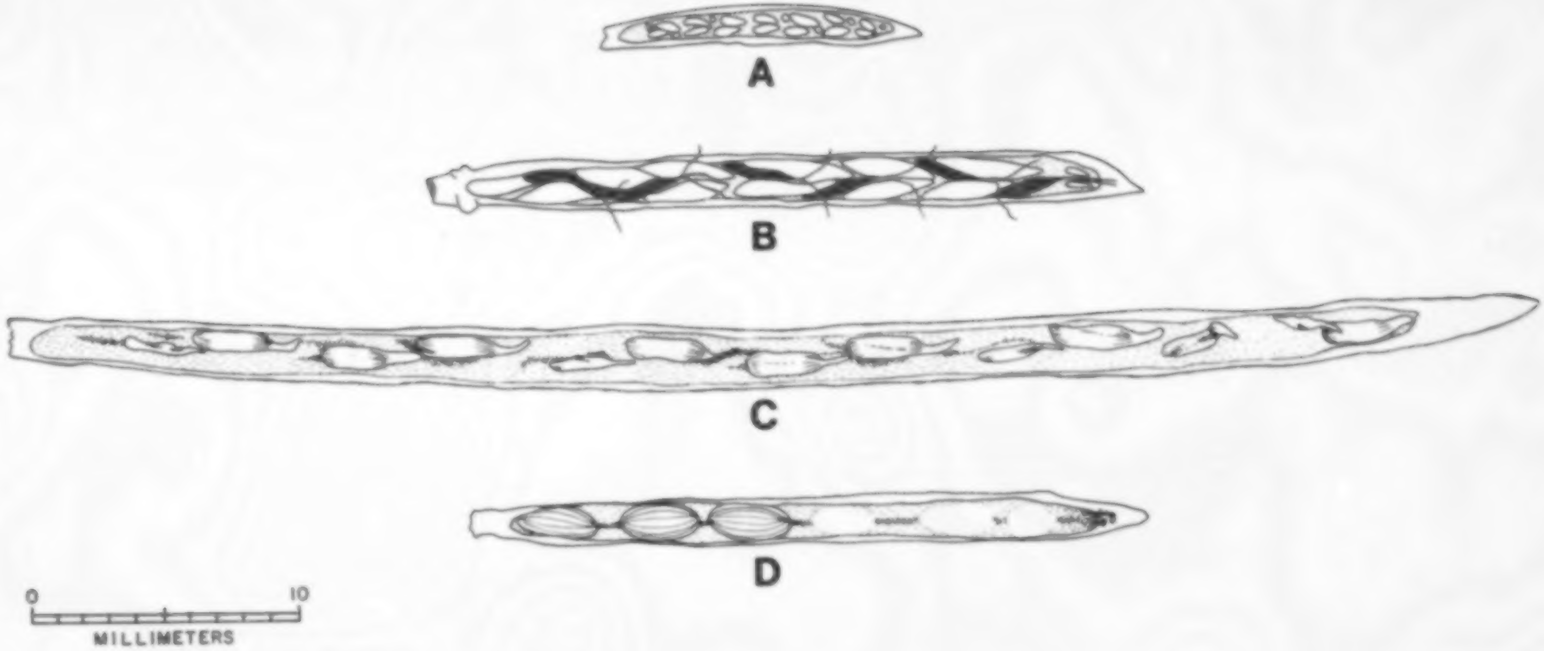

Fig. 2. Phenology of Zostera marina spadices: (a) primordial stage, (b) mature anthers and pistils, (c) fertilized and aborting pistils, (d) mature fruits and seed release.

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2:1 reflecting more developed nature of the spadices. Although the mean number of spadices per shoot was still less than 2 on the 10 April sampling date, additional spadices had developed since March. These were generally smaller in size than the existing spathes resulting in a lower mean number of pollen sacs and pistils per spadice. Along the rhipida the spadices were observed to develop acropetally with decreasing size spathes with increasing branch number as described by Churchill and Riner (1978).

Stigmata loss was first observed in samples taken on 21 April at sites 1 and 2 when water temperatures averaged 16.2°C. The scar tissue at the point of abscission appeared to be reddish brown with the abscission zone located in a slightly swollen area of the style immediately subtending the bifurcated stigmata. The abscissed pistils ranged from 3.7-5.2 mm long. Of the 101 spadices observed, pollen sacs were missing in 14, an indication that pollination had begun. The presence for the first time of fertilized embryos also marked this event. Rapid growth of the reproductive shoots was evident by this time with increases in the numbers of rhipidia per shoot to an average of nearly three. New rhipidia developed basepetally and each new rhipidium consisted of a decreasing number of spadices as compared to the more terminal rhipidia on the shoot. Of the three sites it appeared that site 3, along the Eastern Shore of the Bay, may have been delayed one to two weeks in reaching a developmental stage similar to the western shore sites.

Pollination was complete at all locations by 19 May. Anthers were almost totally absent as evidenced in Table 1. All that remained in the spadices were embryos at various stages of development (striations of the seed coat would be detected) and degenerating unfertilized pistils (Fig. 2c). A few seeds had dehisced as evidenced by pericarp vestiges (Fig. 2d). Most rapid embryo development and corresponding pollen sac dehiscence occurred between 21 April and 21 May at sites 1 and 2, and between 2-28 May at site 3. By 28 May, it was apparent that the fruiting process was at full maturity at all the sites. Water temperatures ranged from 20-21°C during this period. The characteristic markings on the seed coat were obvious and the pericarp of many of the fruits were bursting. Nonviable degenerating pistils were nearly gone and a small number of seeds had been released. The maximum percent of fertilized embryos observed prior to seed release was 59 at sites 1 and 2, and 87 at site 3 (Table 1). Although the mean number of rhipidium per shoot at maximum development in May exceeded three for each of the sites, a range in sizes was observed throughout the beds. Many shoots still consisted of only one rhipidium while the maximum observed was four.

An attempt was made to collect material on 3 June at sites 1 and 2 and on 5 June at site 3. Water temperature was 25°C at this time. However, there was a widespread deterioration of reproductive shoots. Entire shoots were floating at the surface, many of them lacking spadices. Those still rooted had deteriorated as well. The spadices that were present had only a few seeds and seed release was considered essentially complete. Because of these conditions no collections or data were taken.

The maximum density of reproductive shoots collected from each of the sites ranged from 303-424 per m^2 or 11-19% of the total number of shoots (vegetative and reproductive). The mean length of the reproductive shoots

TABLE 1. MEAN NUMBERS OF RHIPIDIA AND SPADICES PER SHOOT, SPADICES PER RHIPIDIUM, POLLEN SACS AND PISTILS PER SPADICE AND SHOOT, AND THE PERCENT OF PISTILS THAT HAD DEVELOPED INTO FERTILIZED EMBRYOS, BY DATE. (1 STANDARD DEVIATION GIVEN IN PARENTHESIS)

	rhipidium·shoot^{-1}	spadices·shoot^{-1}	spadices·rhipidium^{-1}	pollen sacs·shoot^{-1}	pistils·shoot^{-1}	pollen sacs·spadice^{-1}	pistils·spadice^{-1}	% fertilized embryos
Brown's Bay (Site 1)								
3-19	1.0(0.0)	5.0(1.7)	5.0(1.7)	94.0(38.1)	44.0(18.2)	18.8(2.5)	8.8(1.3)	0
4-21	2.7(0.8)	6.5(2.1)	2.4(1.0)	69.2(24.0)	39.3(17.2)	10.6(4.5)	6.0(1.3)	1
5-12	3.4(1.0)	7.2(2.8)	2.1(0.9)	2.5(5.2)	28.6(12.4)	0.4(1.4)	4.0(1.4)	43
5-19	2.9(1.0)	5.7(2.2)	2.2(1.6)	0.0(0.0)	25.6(12.6)	0.0(0.0)	4.5(1.5)	59
Guinea Marsh (Site 2)								
2-14	1.0(0.0)	1.3(0.6)	1.3(0.6)	21.7(12.5)	5.3(4.7)	16.3(2.4)	4.0(4.7)	0
3-19	1.0(0.0)	1.1(0.4)	1.2(0.4)	21.8(4.2)	10.7(1.7)	18.2(3.4)	9.1(1.6)	0
4-21	2.6(1.3)	4.2(2.8)	2.0(1.4)	40.7(29.3)	27.1(20.9)	9.5(5.8)	6.4(1.7)	3
5-12	3.3(0.5)	6.0(1.7)	2.0(0.7)	0.0(0.0)	41.3(10.8)	0.0(0.0)	6.9(1.7)	52
5-19	3.0(0.0)	6.0(0.0)	2.7(0.6)	0.0(0.0)	44.0(0.0)	0.0(0.0)	5.5(1.7)	59
Vaucluse Shores (Site 3)								
2-15	1.0(0.0)	1.5(0.7)	1.5(0.7)	27.5(14.8)	9.5(0.7)	18.3(1.5)	6.3(5.5)	0
3-11	1.0(0.0)	1.0(0.0)	1.0(1.0)	16.7(1.5)	7.3(0.6)	13.3(7.2)	7.3(0.6)	0
3-31	1.0(0.0)	1.3(0.5)	1.3(0.5)	15.4(4.9)	7.0(2.9)	13.6(2.2)	6.8(1.6)	0
4-10	1.1(0.3)	1.6(0.8)	1.5(0.7)	14.5(11.0)	7.2(5.8)	9.9(3.6)	4.5(2.2)	0
5-2	3.1(1.1)	4.1(2.8)	1.8(1.7)	25.0(12.9)	24.6(12.8)	5.6(5.4)	5.4(2.0)	2
5-28	3.3(0.6)	5.7(1.5)	1.7(0.5)	0.0(0.0)	30.0(6.2)	0.0(0.0)	5.3(2.5)	87

ranged from 20.7 cm at the Browns Bay site to 33.6 cm at the Vaucluse Shores site. Assuming that the mean of 68% of the ovaries which had developed into fertilized embryos by late May (Table 1) equaled the percent of seeds produced, populations of *Zostera marina* from the three study sites produced an average of 23 seeds per shoot. Using a mean density of 353 reproductive shoots m^{-2}, an average of 8127 seeds m^{-2} were produced in the Chesapeake Bay *Z. marina* beds.

DISCUSSION

Since Setchell's (1929) classical work on the phenology of *Zostera marina* and his emphasis on the importance of temperature as a controlling mechanism for the different stages in the life cycle of *Z. marina*, numerous workers have compared their phenological data from various localities to either corroborate or refute the original hypothesis (Tutin, 1938; McRoy, 1970; Phillips, 1972; Felger and McRoy, 1975; Harrison and Mann, 1975; Churchill and Riner, 1978; DeCock, 1980; Jacobs and Pierson, 1981). In addition to temperature, irradiance has been implicated as an important factor especially as it relates to floral induction (Backman and Barilotti, 1976; Churchill and Riner, 1978).

The data from our study document the successive development of the flowering process. Initial observations of the immature flowers were obtained in February when water temperatures were 3°C. Completion of the flowering process when mature seeds are released was observed in late May-early June when water temperatures were 23-25°C.

Our data corroborated much of the detailed information on flowering of *Zostera marina* in New York by Churchill and Riner (1978) although some slight differences exist. Data sets from our study and Churchill and Riner's conform, in some respects, to Setchell's (1929) original temperature hypothesis. Setchell suggested that 15°C was the temperature required for anthesis. In the Chesapeake Bay *Z. marina* beds, anthesis was observed when temperatures were nearly 15°C while in New York populations, anthesis started shortly after the water temperature had exceeded 15°C. Setchell as well as Tutin (1938) also suggested that above 20°C, flowers and immature fruits die and slough off the plant. In the Chesapeake Bay, water temperatures were over 20°C for one week before the peak of seed production (10-28 May) with temperatures reaching 23-25°C during the peak of seed production. In New York anthesis occurred primarily while the water temperature fluctuated between 20-21°C. Due to inherent variation in populations of a species along gradients of either depth or latitude, that the differences observed here and in New York may not be significantly different. However, differences between North American west coast populations (e.g. Puget Sound where water temperatures do not exceed 15°C and flowering occurs at 8-9°C (Phillips, 1972), and east coast populations are undoubtedly significant. This latter contrast suggests either temperature adaptation of west coast species or the effect of other factors in floral development.

In our study, we found that the period from initiation of pollen release to initial seed development and release was 28-30 d. These results are similar to the findings of Churchill and Riner (1978) for New York populations. DeCock (1980) also noted a similar length of time for pollen release to seed development, for populations of *Zostera marina* in the Netherlands.

The generative shoots of *Zostera marina* populations studied along the east coast of the U.S. develop in a distinctly different pattern than that reported for European populations. There is basepetal development of rhipidia along the shoot as compared to acropetal and synchronous development found in France (Jacobs and Pierson, 1981) and the Netherlands (DeCock, 1981a), respectively. The size of the generative shoot as well as both the number of spadices and rhipidia is also less along the east coast of the U.S. Although a number of factors including the availability of nutrients and depth (Jacobs and Pierson, 1981) may affect the growth of the shoots, certainly the prolonged periods of favorable summertime water temperatures (maximum water temperature does not exceed 15°C) observed in the European studies is an important factor to consider.

A distinct flowering period combined with the rapid dehiscence of seeds observed in our Chesapeake Bay populations is similar to that observed by Churchill and Riner (1978) for the New York area. In our study, for example, pollination occurred during a three to four week period from mid-April to mid-May. Again, the European studies show distinctly different results with DeCock (1981a) and Jacobs and Pierson (1981) recording prolonged flowering periods.

The question of what ultimately terminates the flowering process has been alluded to in a number of recent papers. Both DeCock (1981a) and Churchill and Riner (1978) speculated that nutrient stress may play an important part in the cessation of flowering. Churchill and Riner (1978) indicated that because of the 20-21°C water temperatures observed during anthesis in their study site it was unlikely that flowering was terminated by unfavorable water temperatures. We submit however that their observations as well as ours suggest that in many areas the *Zostera marina* populations have adapted to different temperature regimes so that flowering may occur at higher limits than the 20°C originally proposed by Setchell (1929). We feel this adaptation could be a particularly important feature in those areas where water temperatures reach or exceed 30°C during the summer. In a similar manner, *Z. marina* populations have been shown to flower in areas where water temperatures never exceed 20°C, suggesting an adaptation to flower at lower maxima (Phillips, 1972; Harrison and Mann, 1975; Jacobs and Pierson, 1981).

Because of the importance of temperature in the life cycle of *Zostera marina* especially the reproductive aspects, latitudinal comparisons of populations should show a progression of stages in the reproductive cycle (e.g. anthesis or seed release) as one moves south. This was initially suggested by Setchell (1929) and later confirmed for the European coast [see Table III, Jacobs and Pierson (1981)]. In addition to the Churchill and Riner (1978) data for New York, we examined the available data for North Carolina (the southern limit of *Z. marina* on the east coast of North America) (Dillon, 1971) and for a Nova Scotian population (Keddy and Patriguin, 1978). We compared events such as first appearance spadix primordia and occurrence of anthesis and mature fruits were noted for each of these areas and the Chesapeake Bay (Fig. 3). The greatest uncertainty in the comparison is associated with observation of the spadix primordia since this determination depends on the frequency of collections and the detail of examinations of each shoot (Dillon did not report on this aspect in his study). Based on the data for these four studies, each reproductive event is reported to have occurred earliest in the most southern location and at successively later dates at more northern sites.

An important question arising from figure 3 is how does the length of the flowering period and the rate at which water temperature increases affect rhipidium and spadix production? We hypothesize that the longer more favorable water temperatures prevail, and the slower the rise of ambient water temperature to summertime maxima, the greater the production of rhipidia and spadices should be. Although data are not available for all of the sites in Fig. 3, Churchill and Riner (1978) do report an average of 7.6 spadices per shoot in New York, which is greater than the averages reported here. Jacobs and Pierson (1981) report an average of 20 spadices per shoot for a Zostera marina population in Roscoff, France where water temperatures, averaging 9-15°C, appear to provide a prolonged, favorable environment for flower production.

Although temperature is an important factor in the timing of the reproductive sequence, irradiance may also be important. Backman and Barilotti (1976), based on the results of their light reduction experiments, suggested the importance of irradiance for flowering while Jacobs and Pierson (1981) noted that irradiance varies with latitude. We suggest that it may share in the timing of Z. marina reproduction. Further experimentation is necessary for elucidation of the relative influence both parameters have on flowering.

In addition to the geographical comparisons of the timing of the flowering processes, these same studies have also compared gross morphological characteristics such as length of flowering shoots, number of flowering shoots m^{-2}, number of seeds produced, etc. (Setchell, 1929; Tutin, 1938; McRoy, 1970; Felger and McRoy, 1975; Churchill and Riner, 1978; Keddy and Patriquin, 1978; Jacobs and Pierson, 1981). Care must be exercised in these comparisons. Morphological characteristics may vary within an area in response to depth, irradiance, nutrients and temperature. Within site variances may therefore actually be greater than between site variances when comparisons are made over latitudinal and longitudinal gradients. Indeed, differences even within one location can vary greatly from one year to the next, thus further complicating comparisons of data sets from short duration studies (one yr or less) and longer term studies. We collected information on the phenology of Zostera marina over a 30-month period at the three sites described here and at several other sites (Orth et al., 1981). Significant differences were found between years for number, length and biomass of reproductive shoots at the study sites, although the timing of flowering events were similar each year.

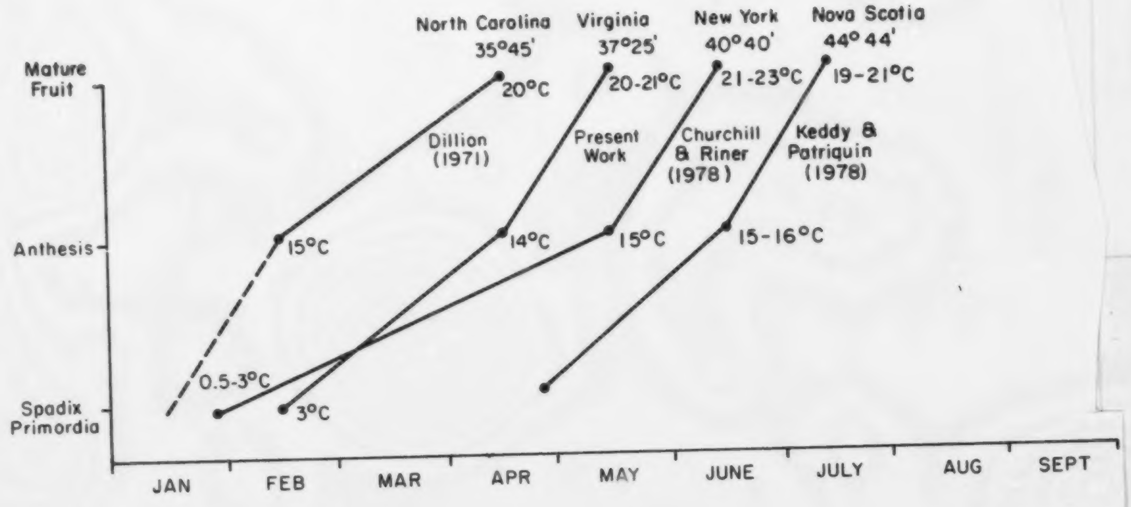

Fig. 3. Reproductive phenology of *Zostera marina* at different locations (with latitude) along the east coast of the United States. The approximate temperature that was recorded for each event is also given (*Keddy and Patriquin provided temperature data for areas that only had an annual form of *Z. marina*. We assumed that these temperatures approximated those that were occurring in nearby beds of perennial *Z. marina*).

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It was interesting to note that at the Vaucluse Shores site in 1979, samples taken along the edge of the grass bed, which was being covered by a migrating sand bar (Orth, et al., 1979), had 26% of the shoots reproductive as compared to 2% and 3% at two nearby sites of similar depths not impacted by the sand bar. This large number of reproductive shoots was observed again in 1980 as well as at another site where sand was also covering the leading edge of the bed. The seagrass bed could be responding to stress (coverage by sand) by producing more reproductive shoots and thus more seeds.

In summary, the reproductive events of Chesapeake Bay _Zostera marina_ populations appear to parallel events described from other study sites. Evidence from our study and other sites along the east coast of North America supports a latitudinal gradient hypothesis based on temperature. However, the importance of irradiance is unstudied and should be a topic for future work. A laboratory approach similar to DeCock's work (1980, 1981a, 1981b, 1981c) certainly suggests this technique as the best and most reliable for examination of the individual and/or combined influences of temperature and irradiance on this aspect of _Z. marina's_ life cycle. We feel that only through a more thorough and rigorous experimental test of these determinants will these hypothesis be accepted or rejected.

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CHAPTER 3

SEED GERMINATION AND SEEDLING GROWTH OF *ZOSTERA MARINA* L.(EELGRASS) IN THE CHESAPEAKE BAY*

by

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ABSTRACT

Seed germination and seedling growth of Zostera marina L. were monitored in the Chesapeake Bay in 1979 and 1980. Harvested seeds were placed in small acrylic tubes at several sites representing the salinity range of Z. marina distribution. Seed germination was first observed to occur first in late September and continue through May with peaks in the fall and spring. The majority of seeds that germinated (66%) did so between December and March when water temperatures ranged from 0-10°C. There was no correlation between sites (different salinity regimes) and frequency of germination rates indicating that salinity was not a major factor in the germination process in this study. Additional information on seed germination was available for seeds collected in 1977 and 1980 and subsequently monitored for germination at only one site. These data were similar to germination frequency recorded in 1979-1980.

Seedling growth was measured from individuals collected from an existing Zostera marina bed. Seedlings were collected from November through May at which time we could no longer distinguish seedlings from existing vegetative stock. Growth was characterized by increased length of the primary shoot, number of leaves per shoot and numbers of shoots per plant. Seedling growth was initially slow during the winter months (water temperature $\leq$ 10°C) but rapidly increased in the spring (temperatures > 10°C). The size range of the harvested seedlings indicated that seed germination in the field probably occurred from October through April, corroborating evidence from the seed germination experiments.

INTRODUCTION

One of the most significant events in the life cycle of seagrasses is the production of seeds. Seed production, and those events related to this process such as flowering, seed release, dispersal, seed germination and subsequent growth of the seedling serve not only as a means of maintaining genetic diversity but also as an important dispersal mechanism. Indeed, dispersal of seeds to an unvegetated area may be the only significant mechanism by which the area can become vegetated. Despite these important functions, there is little information on seed germination in seagrasses and the role that seeds play not only in the maintenance of existing beds but also in the recruitment and re-establishment of new seagrass areas as compared to vegetative reproduction.

Observations and quantitative studies on the biology of *Zostera marina* L. (eelgrass) in many different areas of the world indicate that *Z. marina* undergoes a distinct sexual reproductive phase with the formation of seeds and eventual seed release being the last stages of the flowering process (Setchell, 1929; Taylor, 1957; Churchill and Riner, 1978; Keddy and Patriquin, 1978). De Cock (1980), in particular, provided a very detailed account of the flowering and fruiting of *Z. marina* in the Netherlands. Although there was relatively little known about the fate of the seeds and the seed germination process, previous studies have indicated that, in general, germination of *Z. marina* occurred at lower temperatures (5-15°C) under both light or dark conditions and was higher at lower salinities (10 $^{o}/oo$) than at higher salinities (30 $^{o}/oo$). In addition, there was apparently no dormant period between seed release and seed germination (Setchell, 1929; Tutin, 1938; Addy, 1947; Arasaki, 1950; Phillips, 1972; Orth, 1976; and Churchill, unpublished).

Zostera marina is the dominant seagrass in the Chesapeake Bay and, until recently, was abundant in many of the shoal areas of the Bay and its tributaries (Orth, 1976; Orth and Moore, 1981a, b). Despite its past abundance, relatively little was known on the biology of *Z. marina* in the Bay. Since 1978, a large scale, multidisciplinary research program has been underway on the biology and functional ecology of *Z. marina* in the Bay. One aspect of this research, which is reported here, involved assessing the timing of seed germination and seedling growth of *Z. marina* under natural field conditions. The seed germination process has important implications in the Bay because of the potential use of seeds and seedlings for the re-establishment of recently denuded areas.

Study Sites

Eight sites within and adjacent to the Chesapeake Bay were used during this study for seed germination experiments (Fig. 1). Five sites were

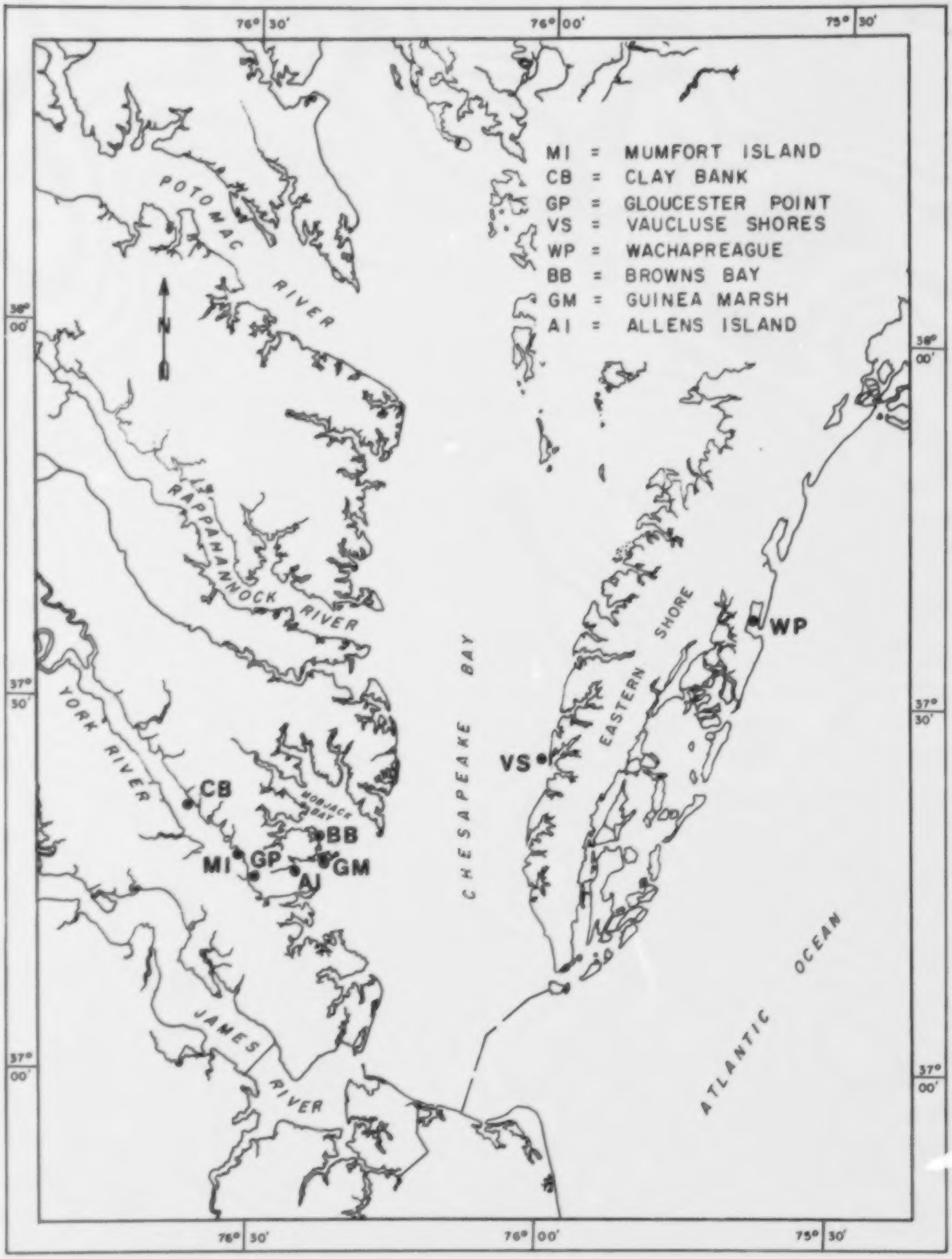
76° 30'
76° 00'
75° 30'
38° 00'
37° 30'
37° 00'
MI = MUMFORT ISLAND
CB = CLAY BANK
GP = GLOUCESTER POINT
VS = VAUCLUSE SHORES
WP = WACHAPREAGUE
BB = BROWNS BAY
GM = GUINEA MARSH
AI = ALLENS ISLAND
N
POTOMAC RIVER
RAPPAHANNOCK RIVER
YORK RIVER
JAMES RIVER
MOBJACK BAY
CHESAPEAKE BAY
EASTERN SHORE
ATLANTIC OCEAN
CB
MI
GP
AI
BB
GM
VS
WP

Fig. 1. Location of field sites used for the seed germination experiments.

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located in the York River, proceeding from the mouth of the York River at the Guinea Marsh site, to Clay Bank, the most upriver limit where Zostera marina formerly occurred. Additional sites were located in Mobjack Bay (Browns Bay) and on the bayside (Vaucluse Shores) of Virginia's Eastern Shore. Table 1 shows the salinity ranges for each of the sites.

The Guinea Marsh, Browns Bay and Vaucluse Shores sites contained dense beds of Zostera marina, with Ruppia maritima (widgeon grass) also co-occurring at the Browns Bay and Vaucluse Shore sites. Allens Island was very sparsely vegetated while the Gloucester Point, Mumfort Island, and Clay Bank sites were unvegetated. The latter three sites and the Allens Island site were densely vegetated with Z. marina in 1973 but the vegetation subsequently declined in 1973 and 1974 (Orth et al., 1979). Z. marina has not been present at the Wachapreague site during the recent past although there is evidence of its presence prior to the wasting disease in the 1930's in the shallows behind the barrier islands near Wachapreague (Orth and Moore, 1981a, b). The Gloucester Point site was used for additional seed germinaton experiments conducted in 1977-78 and 1980-81.

MATERIALS AND METHODS

Seed Collection

Mature seeds, determined by direct observation of developing embryos in reproductive shoots of field populations of Zostera marina and vital staining with tetrazolium red, were collected from established Z. marina beds in May and June, 1979. The method of harvesting involved snorkeling over a Z. marina bed at low tide, removing a reproductive shoot with attached seeds at its base and placing the shoots in a fine mesh collecting bag (0.5 mm mesh). All reproductive shoots from a particular collecting location were transferred to a single nylon mesh bag (0.5 mm mesh) and held in running seawater at our laboratory to allow adequate time for decomposition of the spathe and shoot and subsequent release of the seeds. All material in the nylon bag was washed thoroughly through a 2 mm mesh sieve to separate seeds from most of the other material. Seeds passed through this screen but were retained on a 1 mm mesh sieve. Seeds from each collection were then placed in open, 4 liter containers and held in a running seawater tank until initiation of the germination experiments.

Seeds were collected from three locations in the lower Bay at successive intervals; the mouth of the York River off the Guinea Marshes (May 22, 30 and June 12), Browns Bay (May 14 and 22); and Vaucluse Shores (May 23, 31 and June 7) (Fig. 1). Repeated collections from the sites were made in an attempt to maximize the harvesting of mature seeds. In further discussion of these collections, they will be subsequently referred to in the following notation: Guinea Marsh - GM1, 2, and 3 for each successive collection; Browns Bay - BB1 and 2; Vaucluse Shores - VS1, 2, and 3.

Seed viability of each collection was tested using the vital stain tetrazolium red (2.3, 5-triphenyl-2H-tetrazolium chloride) (Churchill and Riner, 1978). Fifty seeds from each collection were placed in a 0.5%

TABLE 1. SALINITY RANGE FOR EACH OF THE SITES USED DURING THE FIELD SEED GERMINATION EXPERIMENTS.

Site	Salinity o/oo
Wachapreague	25-32
Vaucluse Shores	15-24
Browns Bay	15-20
Guinea Marsh	15-20
Allens Island	15-20
Gloucester Point	14-18
Mumfort Island	12-18
Clay Bank	8-15

solution of the stain. Seeds that exhibited a distinct pink staining of the cotyledon and upper hypocotyl region after 48 hrs. were considered viable.

Seeds were collected at the Browns Bay Zostera marina bed in May of 1977 and 1980 in the above manner, placed in open 4 liter containers and held in a running seawater tank at ambient temperature. These collections will be referred to subsequently as BB 1977 and BB 1980.

Field Germination Tests

Replicate lots of 200 seeds from each designated collection in 1979 were placed in small acrylic tubes (15 cm long, 2 cm inside diameter). Seeds from the following collections were used in the field germination test: VS2, VS3, GM2 and GM3. Perforated plastic caps were placed at each end of the tubes to prevent seeds from washing out; these caps allowed some water exchange with the surrounding medium. The tubes were anchored approximately 5 cm above the sediment surface in water depths of 0.1 to 0.3 m at mean low water (MLW). Tubes were never exposed at low tide. We chose this method of monitoring seed germination as compared to examining large volumes of sediment for germinated seeds in established beds of Zostera marina for several reasons. First, it gave us the ability to use a large number of seeds in a small area. Secondly, it allowed us to repeatedly observe each lot of seeds and to determine within a relatively short period of time (2 weeks or less) when these seeds germinate, and thirdly, it allowed us to observe germination rates in areas with no existing vegetation. Table 2 shows the distribution of the replicate seed lots from the different seed collection periods and the time each tube was placed at the specific location. At the Gloucester Point area, in addition to seeds being located in shallow water, replicate lots of seeds were placed at a second, deeper water area (3 m, MLW).

The seeds in the core tubes at each site were checked at approximately two week intervals for germination. At each sampling period, the tubes were processed immediately on location by placing the contents of each core in a small enamel pan with adequate water and examining the material carefully for germinated seeds. The criterion for successful germination was an extension of the cotyledon from the seed case. Seeds that had germinated were removed from the pan, placed in a holding jar and when returned to the laboratory were located in a running seawater tank. All remaining ungerminated seeds were carefully placed back in the tubes which were then reanchored. No more than 30 minutes elapsed during the sampling procedure.

Seeds from the BB1 and VS1 seed collections were placed in 4 liter jars held in aquaria with running seawater at our laboratory (also located at Gloucester Point) and monitored for seed germination. Seeds collected in 1977 and 1980 were also monitored at our laboratory similar to the BB1 and VS1 collections.

Seedling Growth

In order to estimate seedling growth of seeds that had germinated in established beds of Zostera marina, monthly samples were taken at the Guinea Marsh area from November 18, 1979, to May 19, 1980, for seedlings. Random

TABLE 2. STATION LOCATIONS FOR THE SEED GERMINATION TEST AND DATES WHEN SEED TUBES WERE PLACED AT EACH LOCATION FOR EACH SEED COLLECTION (WHERE TWO DATES OCCUR FOR A COLLECTION, ONE REPLICATE WAS PLACED ON THE FIRST DATE, THE OTHER REPLICATE ON THE SECOND DATE).

	VS2	VS3	GM2	GM3
Browns Bay	++(Aug. 15)	++(Aug. 9)	++(Aug. 9,15)°	+(Aug. 9)
Guinea Marsh	++(Aug. 15)	++(Aug. 6)	++(Aug. 6,15)	+(Aug. 6)
Allens Island	++(Aug. 15)*	++(Aug. 6)*	++(Aug. 6,15)*	+(Aug. 6)*
Gloucester Pt. shallow	++(Aug. 15)	++(Aug. 7)	++(Aug. 7,15)	+(Aug. 7)
Gloucester Pt. deep	++(Aug. 14)	++(Aug. 14)	++(Aug. 14)	
Mumfort Island	++(Aug. 15)	++(Aug. 7)	++(Aug. 7,15)	+(Aug. 7)
Clay Bank	++(Aug. 24)	++)Aug. 10)	++(Aug. 10)	
Vaucluse Shores	++(Aug. 22)	++(Jul. 23)	++(Jul. 23, Aug. 2)	+(Jul. 23)
Wachapreague	++(Aug. 27)	++(Aug. 27)	++(Aug. 27)	

+ - represents one core tube of 200 eelgrass seeds

* - the entire set of core tubes at this site was lost immediately after being placed in the field. Additional core tubes with seeds were set out on Sept. 7. Since there were no remaining seeds from the GM3 collection, this could not be replaced.

° - one seed tube from this collection was lost at the initiation of the experiment and replaced on Sept. 4.

samples of sediment with seedlings and older shoots were collected from the bed and carefully washed free of sediment. Seedlings were identified by the presence of the seed coat which usually was still attached to the primary root, or if the seed was absent, by a scorpioid base, indicative of an older seedling (Setchell, 1929). After the May samples, however, growth of these seedlings had become so vigorous that seedlings could not be distinguished from the previously established vegetative stock. Twenty-five seedlings from each monthly collection were measured for maximum length of the primary leaf (measured from base of cotyledon sheath to tip of primary shoot), number of shoots per seedling and total number of leaves per seedling.

RESULTS

Seed Germination

Monthly and cumulative seed germination data at each of the eight sites (nine collections) are shown in Fig. 2 (seeds from the VS2 collection did not germinate and were not included in these calculations). Water temperature data, superimposed on each graph, were obtained from a continuous temperature recorder located at Gloucester Point. This provided a more accurate representation of temperature variation in the region than spot measurements obtained at each sampling site. These temperature data were used for all sites except Wachapreague, where continuous temperature data recorded from this site were used.

In most cases, the germinating seeds had reached a stage where there was extension of the cotyledon and basal hypocotyl from the seed coat, with various lengths of elongation of the cotyledon. Some individuals had marked extension of the plumule from the cotyledon sheath. No significant development of root hairs on the basal hypocotyl were observed.

Data for seeds germinated (percent and cumulative percent) in the 4 liter jars held in running water for the BB1, VS1, BB-1977, and BB-1980 collections are shown in Figure 3 along with temperature patterns for the seed germination period.

Seeds from the BB2 and GM1 collections in addition to VS2 produced no germinated seeds and were not included in the analysis. Seeds from the GM2 collection had a low germination rate and these data were also not presented here.

There was no significant correlation ($p>0.05$) between the percent germination at the test locations and salinity (Fig. 2) (Spearman's rank correlation test, r_s, Siegal, 1956). Germination of seeds at Clay Bank (38.6%) which is the upriver limit of Zostera marina growth and where salinities averaged 12 $^o/oo$ is only slightly higher than seeds held at the Wachapreague (23.8%) area where salinities average 30 $^o/oo$.

Seed germination in our experiments (including seeds collected in 1977 and 1980) occurred in every month except June, July and August, the three months with highest water temperature. Seed germination was first observed

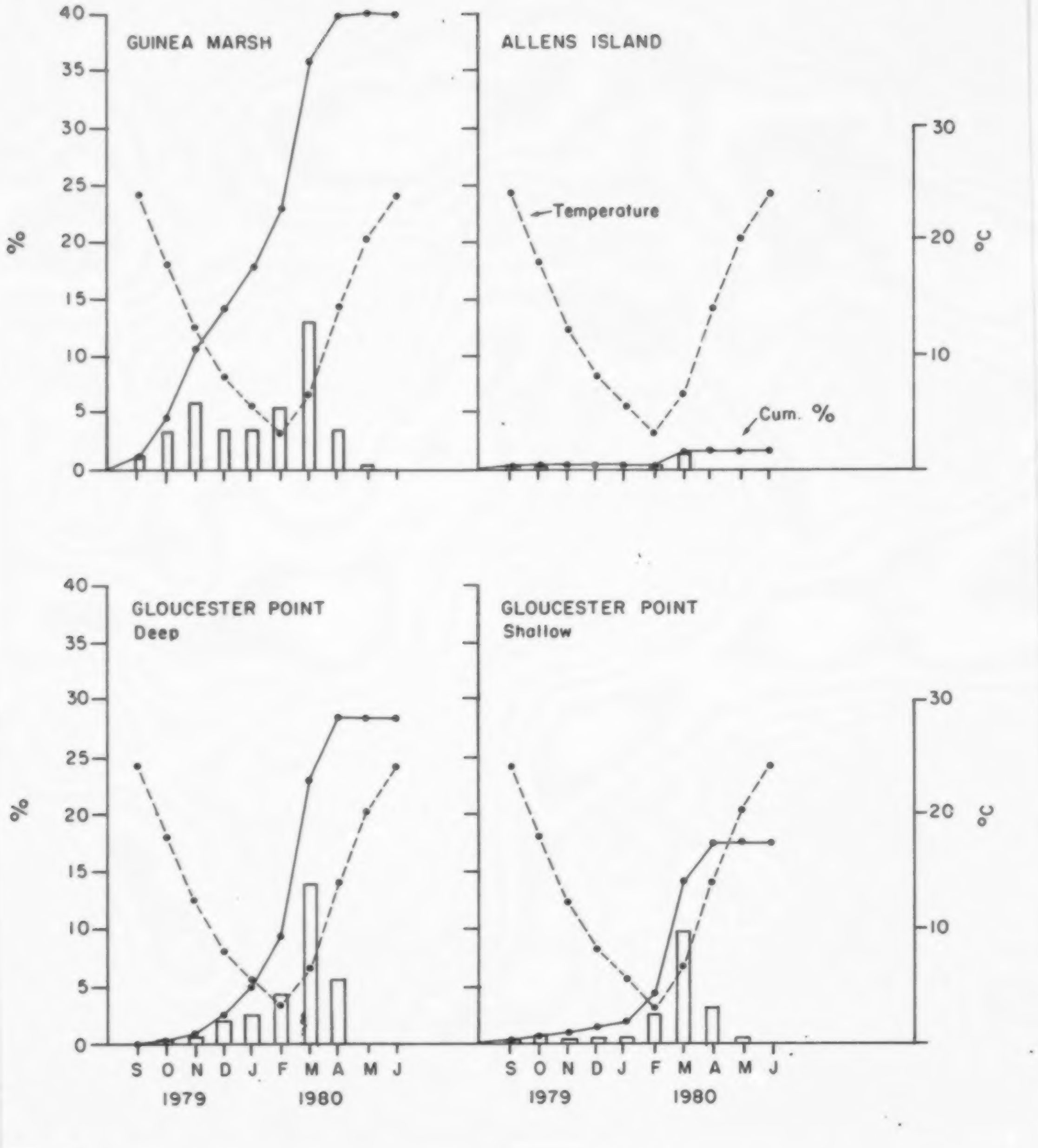

Fig. 2. Percent (bars) and cumulative percent (·—·) of seeds germinating each month from September 1979 to June 1980, plotted against water temperature (·----·) for all field sites.

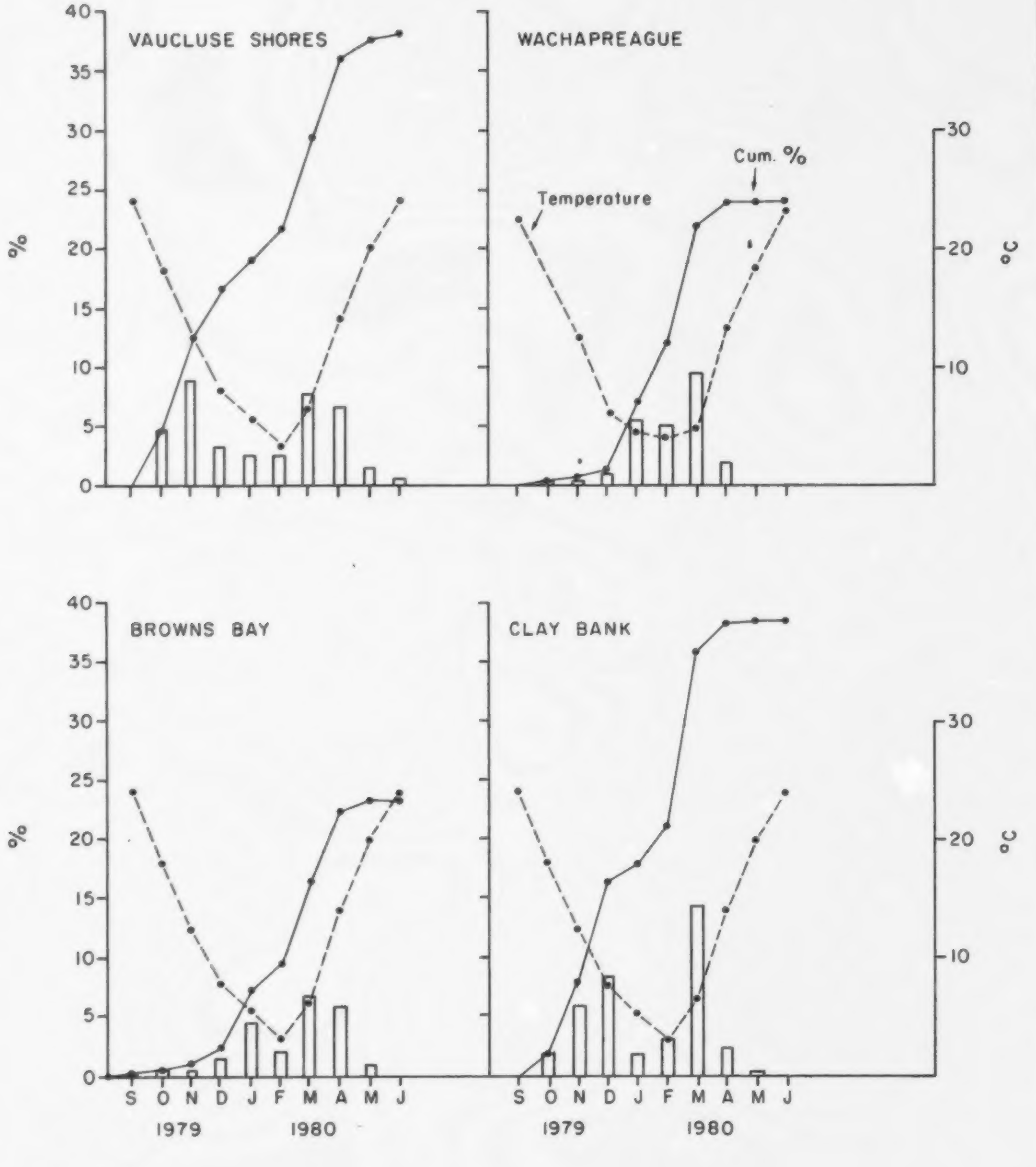

Fig. 2 (continued)

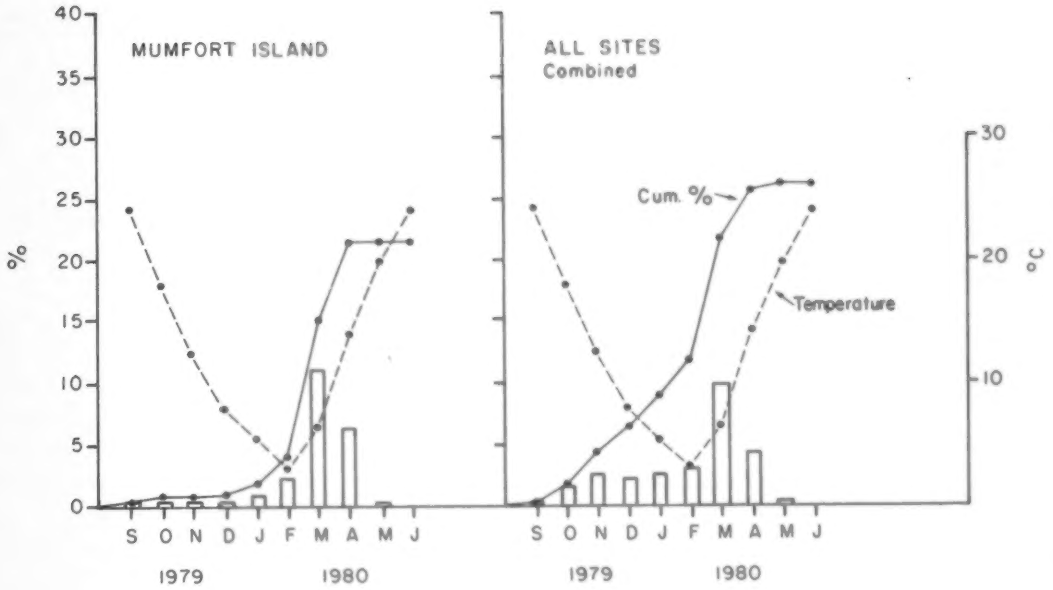

Fig. 2 (continued)

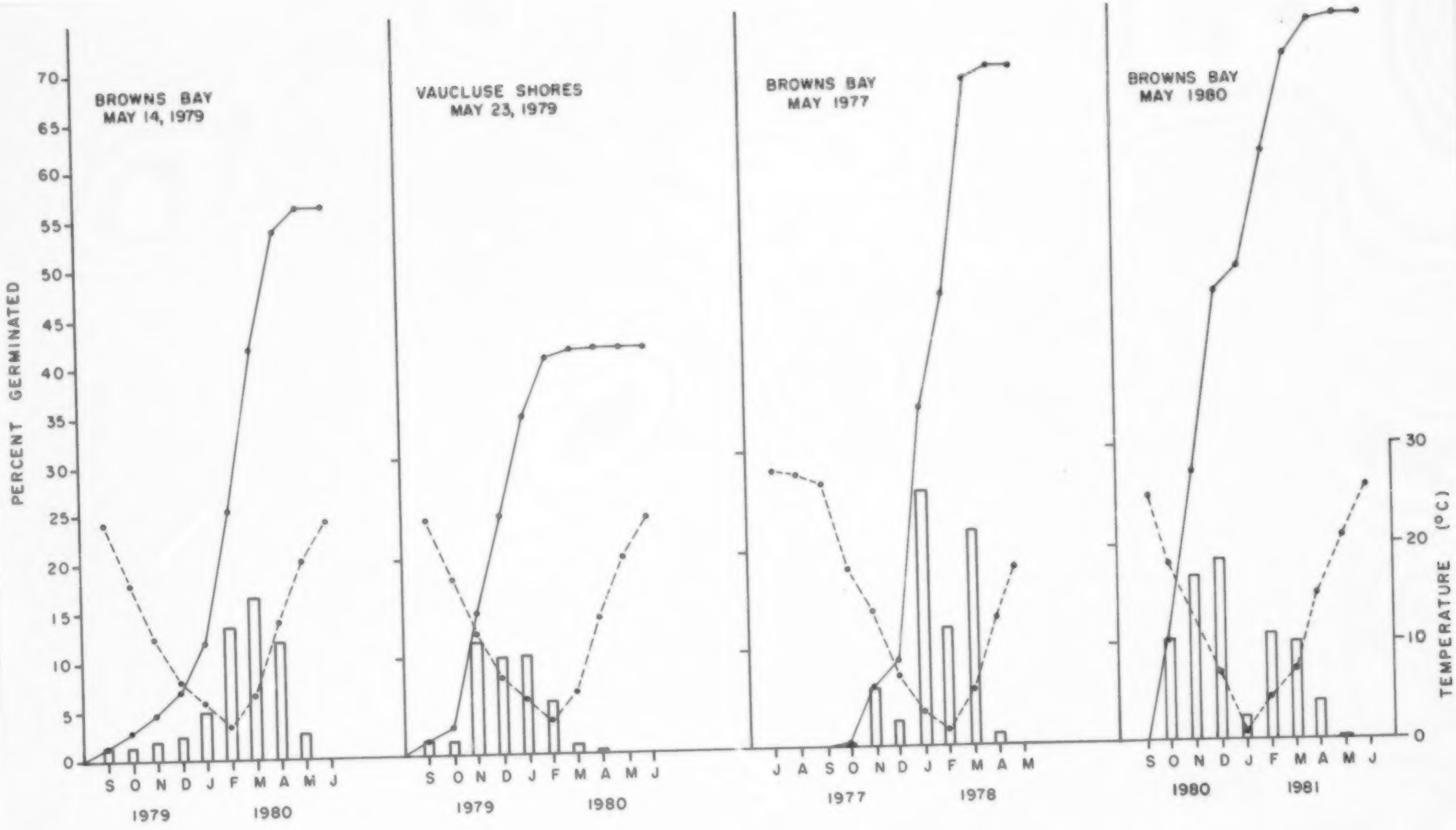

Fig. 3. Percent (bars) and cumulative percent (·——·) of seeds germinating each month from September 1979 to June 1980, plotted against water temperature (·---·) for the two seed collections (Browns Bay May 14 and Vaucluse Shores May 23) held in containers in running sea water at VIMS and two similar collections of seeds taken from Browns Bay in 1977 and 1980.

in a small proportion of the seeds in September and continued through May of the following year. Seed germination in September and May were very low, and at many sites no germination was recorded during these two months.

In the 1979 field test, the major period of seed germination occurred between December 1 and March 31, when 66% of the seeds germinated. Water temperatures ranged from 0 to 10°C during these four months. Every site except Vaucluse Shores had the greatest number of seeds germinate in March (37% of all germinated seeds). Forty-five percent of the total germinated seeds were found through February when water temperatures reached a minimum with the remaining 55% germinating after February as water temperatures increased.

In the remaining two 1979 seed collections and 1977 and 1980 seed collections from Browns Bay, the majority of the seeds that germinated did so in the four month period between Dec. 1 and March 31 (BB1-66%; VS1-65%; BB 1977-90%; BB 1980-57%).

Two trends in the pattern of seed germination were observed. In the first, germination increased initially in the late fall-early winter, declined in mid to late winter and then increased again in the early spring (Vaucluse Shores, Browns Bay, Clay Bank, Guinea Marsh, BB-1977, and BB-1980). In the second, germination was low in the late fall-early winter period, increased in the mid-winter period and reached a maximum during the early spring (Wachapreague, Gloucester Point (both locations), Mumfort Island, and BB1). The VS1 collection was different from all others as 77% of the seeds germinated between November and January. However, when data from all test sites used in 1979 are combined, seed germination was constant through February with March having the highest frequency (Fig. 2).

Seedling Development

Data on the number of leaves and shoots per seedling and the lengths of the primary shoots are given in Figure 4. The initial samples taken on November 28 were most likely germinated in late October or early November, since no seedlings were observed in the area or were present in samples taken in mid-October or earlier. This first evidence of seedlings coincides with the initial period of seed germination observed in our field experiments on seed germination periodicity. By our last sampling date on May 19, the seedlings were difficult to distinguish from vegetative shoots growing from previously established rhizome stock. The lengths of the primary shoots of the seedlings were very similar to the lengths of the vegetative shoots measured for non-seedlings. By June the growth and intertwining of the rhizomes of the seedlings and non-seedling made it impossible to separate one from the other.

Seed germination appeared to be contributing additional seedlings to the area from November to April. During November, 100% of the seedlings sampled could be characterized as being of 8 cm or less in length with only one shoot of two leaves with one primary and two adventitious roots. On the four sampling dates from January to April, respectively, 35%, 35%, 20% and 16% of the seedlings were of similar developmental stage. In November, 68% of the

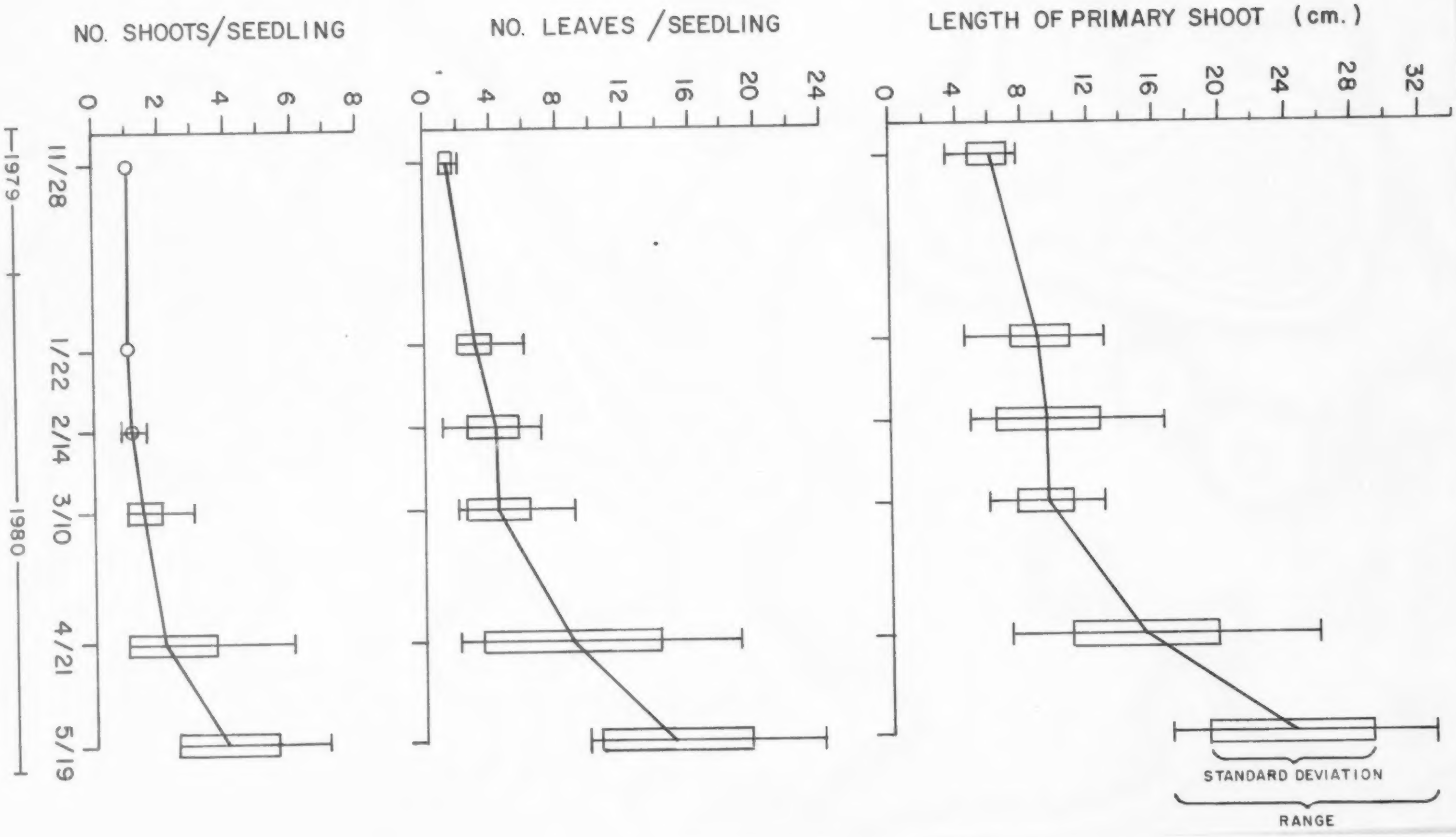

Fig. 4. Mean length of primary shoot, number of leaves per seedling and number of shoots per seedling for seedlings collected at the Guinea Marsh inshore site between November 1979 and May 1980 (mean, range and one standard deviation).

seedlings had their seed coats still attached to their primary roots. From January to April, respectively, 48%, 48%, 24% and 0% of the seedlings were similarly observed.

Growth of older seedlings was apparent throughout the winter period as evidenced by the increasing size range with time of the data presented in Figure 4. During April, for example, although newly sprouted seedlings were still present, the largest seedlings had up to six shoots with over twenty-five leaves. This was the result of possibly five months of growth.

DISCUSSION

In the Chesapeake Bay region, it is evident from both the seed germination experiments and the pattern of seedling development that *Zostera marina* seeds released during May and June begin germinating in the fall, approximately four months later. In addition, this germination process continues throughout the winter into the spring. It also appears that seeds can germinate at least one year after release.

We suggest that the period between seed release and the onset of the germination process in the field is a dormancy induced by high water temperatures. We found virtually no germination when temperatures were above 20°C. Germination was first noted in the fall when temperatures dropped below this level and ended in the spring when temperatures rose again to this point. Germination was most rapid between 5 and 10°C. Our hypothesis is supported by the results of a seed germination experiment conducted in 1977 (unpublished data). Seeds held in 20°C water from September to May never germinated and eventually rotted while 70% of seeds held in seawater subjected to ambient water temperatures germinated during this same period (see Fig. 3, BB-1977 collection).

Additional evidence for the lack of an inherent dormant period other than that induced by high temperatures is available from data on the pattern of seed germination in *Zostera marina* beds along the east coast of the United States. Data from Addy (1947) and Churchill and Riner (1978) and Churchill (unpublished) indicated that there is a decreasing time period between seed release and seed germination with increasing latitude.

In New York (41°,40'N) where seeds are mature and released in July, one month later than in the Chesapeake Bay (37°, 25'N) (Churchill and Riner, 1978; Churchill, unpublished; Silberhorn, et al., unpublished) seeds begin germinating three months after release compared to four in the Bay. In Massachusetts (43°, 40'N) where seeds are released in late July and August, Addy (1947) observed seed germination in the early fall, with little or no dormant period. Although data are not available on seed germination in North Carolina *Z. marina* beds, the most southern limit of *Z. marina* distribution on the east coast of the United States, we could expect a longer dormant period between seed release and seed germination. Seed germination would be expected to begin later in the fall than that observed in the Chesapeake Bay since lower temperatures would occur later than when recorded in the Bay.

Jacobs and Pierson (1981) noted that flowering in European *Zostera marina* beds occurred later in more northern latitudes. Based on these observations and data from the East Coast of the United States presented here, we predict that in European *Z. marina* beds the period between seed release and initial seed germination would decrease with increasing latitude.

Our suggestion that water temperature is the primary variable affecting seed germination is corroborated by data from Long Island Sound (Churchill, unpublished) and additional evidence from the Chesapeake Bay (Orth, 1976). Others, however, have suggested that low salinity is a factor controlling germination (Arasaki, 1950; Phillips, 1972; Lamounette, 1977). Their conclusions are not confirmed by our field experiments where we found no salinity effect among our sites representing the range of salinities where *Zostera marina* has grown or is growing in the Bay (10-25 ^{o}/oo). However, under natural field conditions, the seed germination process probably represents an integration of a number of variables that may act synergistically or antagonistically. Thus, salinity effects may not be expressed with seeds germinating in the field.

The results reported here contrast with earlier work of Orth (1976) and Churchill (unpublished) where seed germination was reported to occur primarily in the fall. There may be other yet unidentified factors that could influence the timing of seed germination, as well as inherent differences between different populations of *Zostera marina*, which may be a result of adaptations to local conditions. It is obvious from the above that only through a more detailed and extensive experimental program studying various combinations of factors, will it be possible to define more accurately the germination ecology of *Z. marina* (e.g. see Van Vierssen, 1982, on the germination ecology of *Zannichelia*).

One of the problems we encountered with our seed germination was the lack of germination in some of the seed collections, e.g. VS2. This may be related to our initial method of holding the reproductive shoots until seeds are released. Those collections which produced small numbers of viable seeds had large amounts of decomposing material packed in the mesh bags. There may have been some factor that affected the viability of the seed in these bags, especially while the shoots decomposed, as the largest seed collections had the lowest germination rates. In order to avoid this potential problem, when collecting reproductive shoots for seeds, we recommend placing them in open, running water systems to allow for adequate water circulation and removing the decomposing shoots soon after seed release.

The length of the primary shoots of the seedlings we observed on November 28 gives an indication of the significant growth that occurs in seedlings in the fall in the Chesapeake Bay region, as the average length was 6.1 cm. We also observed vegetative growth of new shoots from existing rhizome stocks during the first part of October (Orth et al., 1981). Comparisons of plumule (i.e. shoot) lengths of seedlings from Long Island (Churchill, unpublished) and the Chesapeake Bay indicates the more rapid growth of the seedlings in the more southern Chesapeake Bay area as seedlings were larger during comparable time periods. Although Churchill reports little plumule growth between December and March, we observed an increasing number of seedlings with both secondary and tertiary shoots during this period, many over 12 cm in length. Rapid growth in Chesapeake Bay seedlings in the spring resulted in shoot lengths averaging 24 cm as compared to 13 cm in Long Island for the same time period. Churchill does not present temperatures data, but we assume that *Zostera marina* beds in the Bay will experience slightly warmer temperatures than New York *Z. marina* beds during this period. Setchell (1929) reported on seedling growth for plants obtained from Marin County, California from their germination in February through October. Data on changes in leaf length and number of shoots were not presented, but his figures depicting seedling growth were similar to our observations.

Seed germination and the subsequent growth of seedlings can have important implications in not only maintenance and persistence of the existing bed, but also in the re-establishment of denuded areas. Observations and direct sampling of a section of a *Zostera marina* bed at the Guinea Marsh area indicated a substantial contribution to the regrowth of this section by seedlings compared to the production by existing vegetative stock (Orth et al., 1981). In addition, observations of an unvegetated area near an existing *Z. marina* bed at Allens Island showed initial recruitment was extensive and occurred primarily from seeds and subsequent rapid seedling growth after germination. Denuded areas at more distant sites from vegetated areas have not shown any evidence of revegetation, most likely because of the lack of propagules reaching them.

Thus, it appears that the value of the reproductive process in revegetation of denuded areas can be significant. However, the pattern, rate of recovery and analysis of factors controlling this revegatation demand further study.

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CHAPTER 4

TRANSPLANTATION OF _ZOSTERA MARINA_ L. INTO RECENTLY DENUNDED AREAS*

by

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* Results of fertilizer experiment at Allens Island will be published in the Proceedings of the Nineth Annual Conference on Wetlands Restoration and Creation, Hillsborough Community College, Tampa, FL. May 20-21, 1982

ABSTRACT

Zostera marina was transplanted to a total of five sites during 1979 and 1980 along the York and Rappahannock Rivers in areas which contained extensive stands of submerged vegetation prior to 1973. The use of whole plugs including sediments and rhizomes had significantly greater success than a method where shoots were woven into biodegradable mesh and placed on the bottom. Cost per acre were estimated at $8,000 to $42,000 per acre respectively for the two methods using 0.6 m spacing. Survival of the transplants appeared directly related to site location with the most upriver sites having the most rapid and severe failures. Transplants at the donor site adjacent to the existing vegetation had excellent survival using the plug method. Transplantation during the spring, summer and fall seasons, demonstrated best long term survival during the fall, and poorest during the summer. Regardless as to when planted all the transplanted vegetation demonstrated the greatest rate of decline during the months of July and August. This may be related to high water temperatures, increased turbidity and epiphytic growth. Dieback began first in the most upriver locations. The subsurface application of a slow release fertilizer at transplanting time significantly increased both the survival and growth of the transplanted vegetation. A quick release fertilizer had no significant effect.

INTRODUCTION

Zostera marina L. (eelgrass) is the dominant species of vegetation found in the mesohaline, southern half of the Chesapeake Bay (Orth et al., 1979). Along with a companion species *Ruppia maritima* (widgeon grass), this species forms extensive meadows throughout many portions of the lower Bay shoreline and its major tributaries (Orth et al., 1979). These *Z. marina* dominated beds, as with other submerged grass systems throughout the world, are considered an important component of estuarine and coastal ecosystems (Phillips, 1974a; McRoy and Helfferich, 1977; Thayer and Phillips, 1977; Phillips and McRoy, 1980; Phillips, 1980a, 1980b).

In addition to their resource value, the physical presence of seagrass beds helps to stabilize sediments and protect the adjacent shorelands from erosive events (Ginsburg and Lowenstam, 1958; Zieman, 1972; Eleuterius, 1975). The importance of a seagrass bed as a preventive mechanism for substrate erosion has best been shown in areas where the seagrass bed was removed. Wilson (1949) and Rasmussen (1973) describe shore conditions before and after the demise of *Zostera marina* in the 1930's. Wilson (1949), working in England, indicated a lowering of ground level of 2 feet or more due to the erosion of the sand where the *Z. marina* had died. A stone layer beneath the original sand layer became exposed after the removal of grass and sand, and was colonized by seaweed. Rasmussen (1973), working in Denmark, showed that beaches covered with *Z. marina* underwent similar changes. There was a general lowering of the shore with exposure of a stone layer and coarser sediments prevailing where fine sediments once dominated. The disappearance of *Posidonia* beds in France due to pollution (Maggi, 1973) resulted in extensive erosion of the bottom substrate and shoreline. A shell layer which was under the grass bed eventually became exposed and was washed shoreward. There was a loss of 15 cm to 30 cm of sediment in an area in the York River, Virginia, where *Z. marina* was removed by cownose ray activity (Orth, 1975).

Although submerged vegetation can in many cases absorb some extreme environmental events such as hurricanes (Oppenheimer, 1963; Thomas et al., 1961), they are susceptable to both natural and man made perturbations (Duncan, 1933; Renn, 1936; Odum, 1963; Thayer et al., 1975; Orth, 1975; Orth, 1976; Rasmussen, 1973, 1977; Phillips, 1980b). Increased utilization of the coastal zone, especially in the United States, has led to increased demands to be placed upon existing beds of submerged aquatic vegetation and subsequently a desire to ameliorate or mitigate losses of vegetation where possible.

In recent years there has been a resurgence of interest in transplantation of seagrasses. Earliest documented efforts (Addy, 1947a,b) were prompted by a desire to restore areas of *Zostera marina* that had been greatly reduced by the "wasting disease" phenomenon of the early 1930's (Tutin, 1938). More recently efforts have been concentrated on transplanting several dominant species of vegetation, Z. marina, Halodule wrightii, Thalassia testudinum, and Syringodium filiforme. Studies have investigated different procedures for transplanting and anchoring these seagrasses (Kelly et al., 1971; Phillips, 1972; van Breedveld, 1975; Thorhaug and Austin, 1976; Phillips, 1974b; Phillips, 1980a,b), as well as attempting to mitigate actual losses of vegetation caused by dredging and other bottom disturbing activities (van Breedveld, 1975; Robilliard and Porter, 1976; Churchill et al., 1978; Phillips et al., 1978; Fonseca et al., 1979).

Although there have been a number of studies dealing with the transplantation of marine grasses there has been no recently reported work in the lower Chesapeake Bay region, especially in areas once dominated by Zostera marina. A recent study of the distribution and abundance of submerged vegetation in the lower half of the Chesapeake Bay and its tributaries (Orth et al., 1979) has confirmed earlier observations (Orth and Gordon, 1975; Orth 1976) that there has been a considerable decline in vegetation in many areas since approximately 1973. Losses of vegetation have been particularly severe within Virginia's tidal tributaries, especially the York, Rappahannock and Potomac rivers where large beds dominated by Z. marina that previously extended up to 30 km from the river's mouths are now completely gone. This decline apparently occurred within a two year period and at the initiation of this study in 1979 there appeared no evidence of natural revegetation.

Because of the value of the submerged grasses, and the lack of natural revegetation of these denuded areas and interest by the public in transplanting grasses into the barren areas, this project was proposed to assess the feasibility of transplanting wild plants of Zostera marina using existing techniques in order to revegetate selected pilot areas within Virginia's tidal rivers. Factors such as time of year of transplantation, location and depth of sites, survival and growth of transplants, and effects of fertilizers on success were to be investigated. In addition to its value as a management tool, transplantation of Z. marina into presently denuded regions can provide insight into limiting factors controlling the natural revegetation of these areas and indicate whether the original declines may have been due to episodic or chronic conditions.

MATERIALS AND METHODS

Spring Transplanting Effort (1979)

The initial transplantation effort began in March 1979. The primary goal of this effort was to test the feasibility of transplanting Zostera marina in the Chespapeake Bay using two methods for transplanting whole plants (Thorhaug and Austin, 1976; Addy, 1947; Phillips, 1974, 1980; Fonseca and Kenworthy, 1979). In the first method, developed by Fonseca and Kenworthy (1979) and successfully utilized in a mechanically disturbed Z. marina area in North Carolina in the fall of 1978, whole plants were removed by shovel from an established bed located at the Guinea Marsh area near the mouth of the York River in Virginia. The plants were transported in water to the lab where vegetative shoots with the associated sections of rhizomes were separated from the reproductive shoots. The rhizomes with the attached vegetative shoots were then woven into precut 20 cm x 20 cm squares of biodegradable marsh paper (Holdgro manufactured by Gulf States Paper Corp., Alabama) at a density of approximately 10 shoots per square and stored in running seawater until planted.

In the second method, 10 cm diameter plugs including whole plants, roots and rhizomes and associated sediment to a depth of 10-15 cm, were removed by the use of plastic coring tubes from the same established grass bed. The individual plugs were immediately placed in small plastic bags and stacked in insulated plastic coolers for transportation to the transplant site. Wet burlap was layered with the plugs to keep the *Zostera marina* shoots moist.

The transplant site selected to receive both the plugs and mats was located in the Mumfort Island area of the York River (Fig. 1) approximately 13 km upstream from the river's mouth. This area was selected for the following reasons: until 1973 it was the site of extensive eelgrass beds (Orth et al., 1979) and was the location of intensive studies on the epifauna and infauna of *Zostera marina* beds (Marsh, 1970, 1973, 1976; Orth, 1973); the area is presently devoid of *Z. marina* (Orth et al., 1979); the site is relatively isolated and mainimum human disturbance was expected.

At the Mumfort Island site a total of eight treatments were used in transplanting (Table 1). Each treatment consisted of 42 mats or plugs of *Zostera* arranged in a 6 x 7 array with two foot (61 cm) centers (Fig. 2). Two locations were selected within what had been determined by archival aerial photography to be the previous bed outlines. The first was an inshore area approximately 150 m from the largest island (depth, 0.5 m at MLW) and the second an offshore area 300 m from the island (depth, 1.0 m at MLW). These are representative of the depths at which *Z. marina* is generally found around the lower Chesapeake Bay (Orth et al., 1979). At each location the four treatments consisted of two arrays of plugs and two of mats, one of each method fertilized at planting with commercially available ammonium nitrate (34-0-0) and one left unfertilized.

The plugs of *Zostera marina* were implanted by overlaying a large (10 x 12 ft) grid on the shallow bottom to locate the planting sites. Using a coring tube, a 10 x 15 cm plug of sand from the unvegetated bottom was removed at the appropriate 2 ft (61 cm) spacing, 25 g of fertilizer added into the hole (for fertilizing treatments only) and the plug of *Z. marina* with roots and rhizomes and attached sediment inserted. Care was taken to insure that the *Z. marina* was planted at the correct depth. Each plug was then marked with a small orange stake.

Each *Z. marina* mat was placed on the bottom at the correct spacing and anchored into the sediment with U-clips. In the fertilized treatments, 25 g of fertilizer were spread over each of the mats. Finally, each mat was marked with a small orange stake.

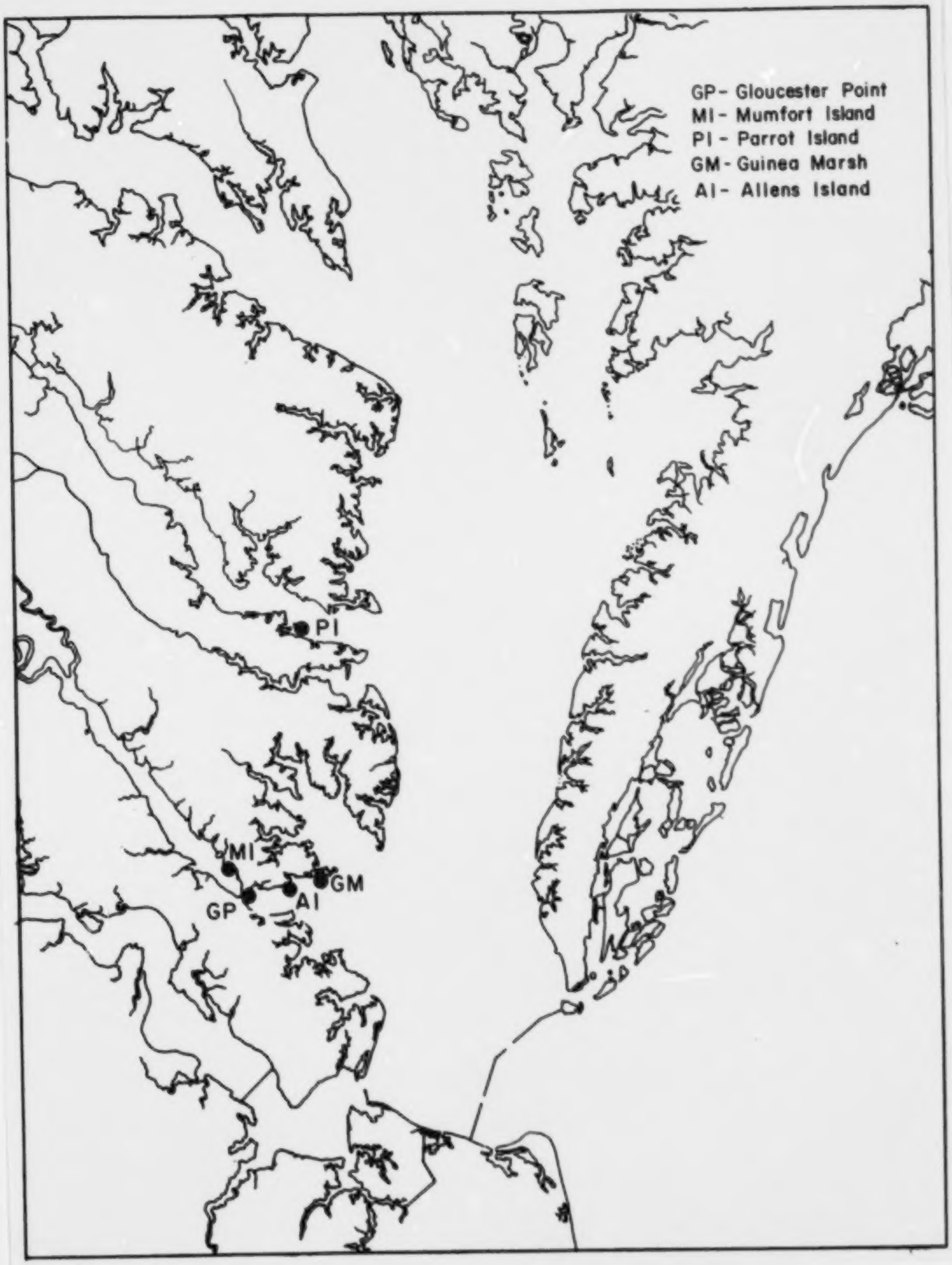
GP- Gloucester Point
MI - Mumfort Island
PI - Parrot Island
GM - Guinea Marsh
AI - Allens Island
PI
MI
GM
GP
AI

Fig. 1. Map of lower Chesapeake Bay showing locations of transplant sites.

TABLE 1. SUMMARY OF ZOSTERA MARINA TRANSPLANT EFFORTS

Date	Treatment	Location
Spring 1979	Plugs unfertilized	1 site, 2 depths (0.5 m, 1.0m @ MLW)
" "	Plugs fertilized	1 site, 2 depths (0.5 m, 1.0 m @ MLW)
" "	Mats unfertilized	1 site, 2 depths (0.5 m, 1.0 m @ MLW)
" "	Mats unfertilized	1 site, 2 depths (0.5 m, 1.0 m @ MLW)
Summer 1979	Plugs unfertilized	4 sites, 1 depth (0.7 m); 1 site, 2 depth (0.5 m, 1.0 m)
" "	Plugs fertilized	4 sites, 1 depth (0.7 m); 1 site, 2 depth (0.5 m, 1.0 m)
Fall 1979	Plugs unfertilized	5 sites in October, 1 depth (0.7m); 1 site in September 1 depth (0.7m)
" "	Plugs fertilized	5 sites in October, 1 depth (0.7m); 1 site in September 1 depth (0.7m)
Spring 1980	Plugs unfertilized	4 sites, 1 depth (0.7 m)
" "	Plugs fertilized	1 site, 1 depth (0.7 m), 2 types of fertilizers

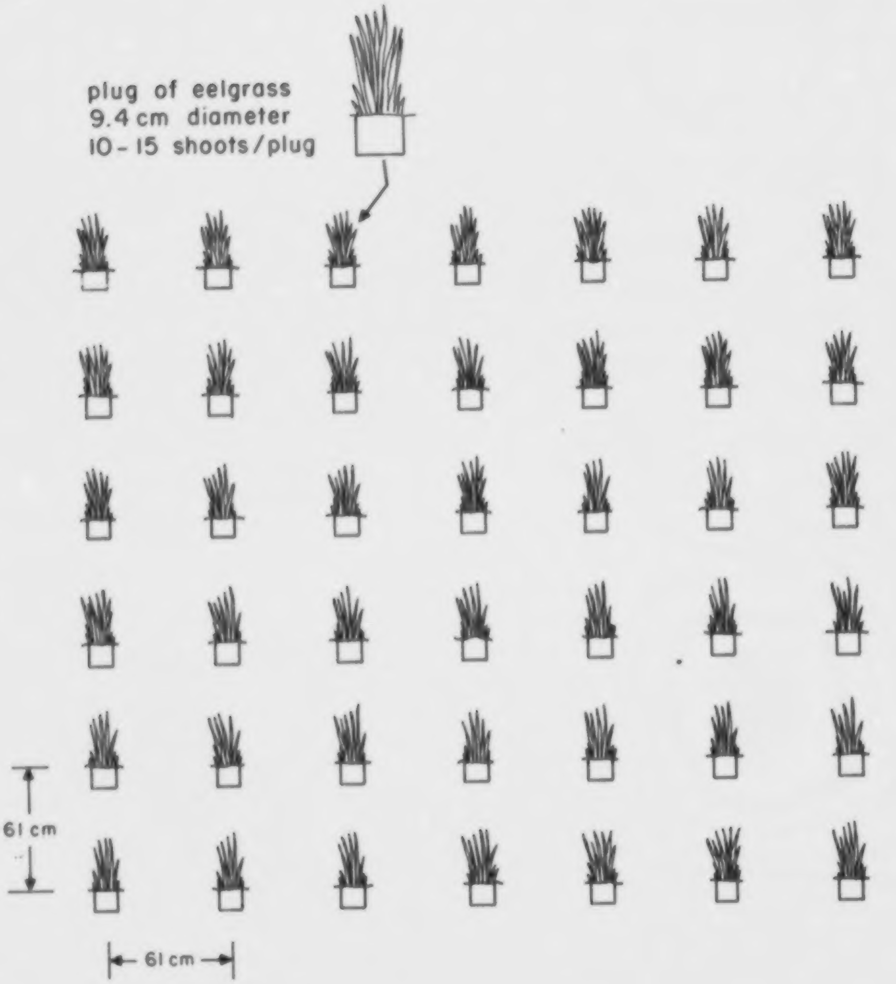
plug of eelgrass
9.4 cm diameter
10 - 15 shoots/plug
61 cm
61 cm

Fig. 2. 6 x 7 array of _Zostera_ _marina_ plugs.

For comparison of the time and effort needed for each of the methods accurate records were maintained of the man-hours required for transportation to or from the sites. This included digging up the stock and obtaining the cores, preparing the mats, and planting the mats and plugs. The transplanted material was then qualitatively observed for growth and survival of transplants. Temperature, salinity and secchi disk measurements were routinely made.

Summer Transplanting Effort (1979)

A second transplanting effort was initiated in early June (Table 1). It coincided with increasing water temperatures (15 to 20°C) and near maximum standing stock of *Zostera marina*. Based on results obtained from the spring transplanting effort, several changes were made. First, only the plug method of transplanting was undertaken. Second, in addition to the Mumfort Island location four other sites received *Z. marina* transplants. Three of these sites were located along the northern shoreline downriver from the Mumfort Island area while one was located along the Rappahannock River. The first additional site was at the Guinea Marsh area (Fig. 1) immediately adjacent to where the donor plugs were obtained at the mouth of the York River. This site consisted of a large patch of unvegetated sandy bottom surrounded by a dense *Z. marina* bed. The second site was located approximately 5 km upriver, and was adjacent to an area of *Spartina alterniflora* dominated marsh known as Allens Island. Until 1973 this shallow littoral area was heavily vegetated with *Z. marina* but today only a few isolated patches of grass remain adjacent to the island's shoreline. A third area was located near the VIMS laboratory at Gloucester Point approximately 5 km upstream from Allens Island and 5 km downstream from Mumfort Island. Here too, a dense bed of *Z. marina* recently existed (1973) but today no vegetation is found. Approximately 2 km downriver from Gloucester Point the most upstream patches of *Z. marina* are currently found. A fourth transplanting site was located in the vicinity of Parrotts Island on the Rappahannock River. As with the other sites it was vegetated with *Z. marina* until the early 1970's, but today is devoid of submerged aquatic vegetation.

At each of the four new transplanting sites only one depth zone was planted. This was approximated 0.7 m below MLW and represented the median depth at which *Zostera marina* is found around the lower Chesapeake Bay. Both the 0.5 m and 1.0 m below MLW zones were continued at the Mumfort Island site.

Each of these six locations (two at the Mumfort site, one at each of the rest) received two treatments of 6 x 7 arrays of *Zostera marina* plugs. One treatment was fertilized with ammonium nitrate and one left unfertilized. The plugs were transplanted in a manner identical to that described for the spring transplanting effort. Growth or decay of transplants was followed by monitoring percent survival of plugs as well as the numbers and lengths of shoots in the surviving plugs. Temperature and salinity measurements were made at each visit to the sites as were secchi disk readings when water depths were greater than the secchi depth.

Fall Transplanting Effort (1979)

A third transplanting effort was initiated in September and October 1979 (Table 1). This period was chosen to correspond to decreasing water temperatures (25 to 20°C), and increasing water clarity (secchi 1.0 to 1.7 m). In addition, it occurred after the annual late summer period of senescence of *Zostera marina*, which is characterized by high water temperature, high turbidity (secchi 0.5 to 1.0 m) and heavy epiphytic growth on the *Z. marina* leaves.

Methods employed in this fall transplanting effort followed very closely those utilized during the summer period. At one depth at each of five sites (Guinea Marsh, Allens Island, Gloucester Point, Mumfort Island, Parrott River) two 6 x 7 arrays of *Zostera marina* plugs were transplanted. One treatment was fertilized with ammonium nitrate and one was unfertilized. The plugs were transplanted in a manner identical to that of the spring and summer transplants and located immediately adjacent to the existing summer, 1979 arrays. Growth or decline of the transplants was followed by monitoring percent survival of total number of plugs as well as the number and length of turions in the surviving plugs. All five areas were transplanted in mid-October, 1979. The Allens Island site was transplanted with two additional fertilized and unfertilized arrays in mid-September 1979 to further investigate an optimum time for transplanting *Z. marina* in this region of the Chesapeake Bay. Temperature, salinity and secchi disk readings were routinely obtained.

Spring Transplanting Effort (1980)

A fourth transplanting effort was initiated in April 1980 (Table 1). This period was chosen for comparison with the spring 1979 transplanting effort and corresponded with increasing water temperatures and rapid growth of *Zostera marina*. All four sites along the York River (Guinea Marsh, Allens Island, Gloucester Point, Mumfort Island) were transplanted with unfertilized, 6 x 7 arrays of *Z. marina* plugs at one depth (0.7 m) which were placed adjacent to previously transplanted (Fall, 1979) arrays. The Parrotts Island site on the Rappahannock River was omitted from this effort in order to concentrate investigations on the range of sites available on the York.

As a result of the findings of the 1979 transplanting effort, the number of fertilized transplants were reduced in spring 1980. However, to continue the investigations of the effect of fertilizers on the survival and growth of transplanted *Zostera marina* as well as to investigate the fate of these fertilizers after application, several studies were initiated.

In March 1980 replicate sediment cores were obtained prior to transplantation in the unvegetated bottom at each of the four transplant sites along the York River (Guinea Marsh, Allens Island, Gloucester Point, Mumfort Island) as well as the vegetated donor site at the Guinea Marsh. These cores were obtained to compare the donor and recipient sites for any differences in existing sediment-nutrient regimes.

The cores were obtained by use of 5.0 cm (2") O.D. plexiglass core tubes 50 cm in length which were graduated on the side in cm. The tubes were forced into the bottom to a depth of approximately 30 cm in the center of the plugs, plugged with a rubber stopper and pulled from the bottom with the core tube containing the sediment vegetation (if present) and the overlying water. The individual tubes were capped at the bottom and placed in a covered container filled with ambient temperature seawater. Immediately after all the samples were taken the core tubes were returned to the lab within 30 minutes for extraction.

Upon return to the laboratory an individual core tube was unplugged and an aliquot of the overlying water extracted using a large hypodermic syringe with an attached 0.4 μ glass fiber filter in a filter holder. The filter was placed in a 50 ml plastic, conical certrifuge tube with screw cap and immediately frozen for later analysis. The sediment plug was extruded from the core tube onto a graduated holder and sectioned into 0-2, 2-5, 5-10 and 10-15 depth segments. Each section of plug sediment was placed in a Gelman filter-centrifuge tube holder and centrifuged through a 0.45 μ glass fiber filter to extract the pore water. The filtered pore water was transferred to a 50 ml capped centrifuge tube and immediately frozen. The remaining sediment fraction was frozen for later grain size analysis according to procedure outlined in Folk (1961).

Pore water from each segment and the sample from the overlying water were analyzed for NH_4^+, NO_3^-, N_2^- and PO_4^{-3} using automated analysis techniques (EPA, 1974) with a technitron auto-analyzer. Modifications to these techniques were made after Wetzel et al., 1979, including concentration of nitrate/nitrite reagents, a two reagent chemistry for phosphate determination and a two reagent chemistry for ammonia (Solorzano, 1969; Koroleft, 1970; Gravitz and Gleye, 1975; Liddicoat, Tibbits and Butler, 1975).

In a similar manner half (21) of an unvegetated array of plugs at the same location were fertilized with ammonium nitrate and half (21) with Osmocote. In this treatment 10 cm plugs of sediment were removed from the bottom, 25 g of fertilizer were added, and the sediment plugs were replaced in the same hole. The unvegetated plugs were then marked with small stakes for later sampling.

At T (date of transplant) + 10 days, T + 37 days, two sediment cores were obtained in each of the six treatments at the Allens Island site: *Zostera marina* plug + ammonium nitrate; *Z. marina* plug + Osmocote; *Z. marina* plug unfertilized; bare sediment + ammonium nitrate; bare sediment + Osmocote; bare sediment unfertilized.

Growth or decline of the transplants were followed as in previous transplanting efforts by non destructive sampling methods for a period of 216 days by SCUBA or snorkel. At every sampling period each plug was examined for percent survival of the total number of plugs (undisturbed by the nutrient sampling), numbers and densities of turions in the surviving plugs, number of reproductive shoots and areal spread of the plugs. Temperature and salinity measurements were made at each visit.

Light attenuation was measured by use of a Li-COR PAR meter with cosine collector beginning in June, 1980. Three to five PAR measurements were made per day from 0800 to 1600 hours EST at each of the transplant sites at approximately weekly intervals. Sampling runs initiated with the most downriver site and proceeded upriver to minimize intersite tidal stage variation. Days when high or low slack periods approximated 1200 hours were preferentially chosen. At each station light readings were obtained at 0.25 m intervals from the surface (just above the water's surface) to bottom (15 cm above bottom). The attenuation coefficient (Kd) was determined using the surface and bottom readings. Kd was calculated by the function:

$$Kd = \frac{-\ln \frac{E_2}{E_1}}{(Z_2 - Z_1)}$$

where ln is the natural log, E_2 is the irradiance at depth Z_2, E_1 is the irradiance at depth Z_1 and $(Z_2 - Z_1)$ is the distance between the two depths in meters. The units of Kd are m^{-1}.

At the Gloucester Point site an attempt was made to assess the impact of the mud snails (*Ilyanassa obsoletus*) which were observed in the spring of 1979 to completely cover the transplanted plugs at the site. Replicate $1m^2$ cages covered with 6 mm mesh screening but open at the top and bottom were each placed around four transplanted plugs in March prior to the snail infestation for approximately a three month period. They were regularly cleaned of epiphytes and those few snails which managed to get inside the exclosures were removed. Comparisons of the growth and survival were made between the caged and the unprotected plugs.

RESULTS AND DISCUSSION

Spring 1979 Transplanting Effort

Transplantaion of four 6 x 7 arrays of *Zostera marina* (168 transplants) by use of mats required 82.5 man hours of effort. In contrast, the transplantation of an equal number of plugs required 16.0 man hours. These equate to 2.0 transplants per man hour for the mat method and 10.5 transplants per man hour for the plug method. Both of these time and effort measurements included all aspects of the transplantation process excluding transportation time from the donor to the recipient site. Obviously the plug method proved much more time effective than the mats. Most of this time differential resulted from the tedious steps of having to weave the individual *Z. marina* plants into the mat fabric. Planting time for each method proved to be about equal, while harvesting the individual plugs required more time than digging up and washing clusters of shoots with entangled roots and rhizomes for the mat method.

Churchill et al. (1978) provide comparative time and effort data with their use of miniplug transplants near Long Island, New York. In their study, 26.6 miniplugs were transplanted per man hour from sites less than one mile apart, so as to effectively negate transportation time. Other studies (Ranwell, 1974; Fonseca et al., 1979; Phillips, 1980) provide more difficult comparisons because data is provided in cost per area and costs of labor and vessels vary from study to study and from year to year. Fonseca et al. (1979) provide a table listing cost comparisons for different methods of transplantations of Zostera marina for several published studies which range from $0.009 to $0.27 cost per shoot. We calculate for our study using rates of $5 per hour wages and $100 per day boat rental (the same costs as Churchill et al. 1978) that our plug method would cost $0.07 per shoot and our mat method $0.38 per shoot for 10 shoots average per mat or plug. Fonseca et al. (1979) report costs of $0.086 per shoot for their comparable mat method and projected costs of $0.028 by using improved weaving techniques. The used an average of 15 shoots per mat.

Phillips (1980b) lists comparative costs of several published studies which range from $1,645 to $76,545 per acre although the data used for the latter figure has been questioned (Fonseca, personal communication). The densities of the transplants vary greatly from study to study however. We estimate costs per acre of approximately $8,000 and $42,000 per acre using 0.6 m spacing for the plugs and mats, respectively. This compares with Churchill et al. (1978) cost of $3,370 per acre using mini plugs in Long Island.

In addition to the time advantage of the plugging method in this study the plugs themselves with the associated sediment provided a stable anchor for the Zostera marina plants in the highly exposed Mumfort Island location. The Z. marina plants woven into the mesh of the mats, were hard pressed to remain in position during periods of increased wave activity. Fonseca et al. (1979) found that the mesh mats survived quite well after a fall 1978 transplanting in a Z. marina area near Beaufort, North Carolina. However, their site was much more protected than the Mumfort Island site in Virginia and was surrounded by an existing Z. marina bed.

Water temperature varied from 10 to 15°C during this transplantation effort in late March. Initially all the plugs and mats appeared to be doing quite well with an apparent difference between the fertilized and unfertilized treatments. After a week however, the mats at both the 0.5 m and 1.0 m depths at Mumfort Island began to be ripped apart by the high energy of the waves and many of the individual turions were lost. The Zostera marina plants trnsplanted in the plugs were much less affected by storm waves and only a few shoots were lost. By mid-April it was apparent that the mats were not holding up well. Not only were they affected by the tidal currents and wind waves, but several were uprooted by the burrowing of bluecrabs (Callinectes sapidus). The plugs were also affected by the blue crabs and several were lost by this burrowing activity. The habitat values of these small areas of plants became obvious as the small transplants immediately attracted numerous crabs, small fish and snails.

Churchill et al. (1978) similarly reported a greater loss of miniplugs (shoots, roots and rhizomes with no sediment) when they compared them to plugs with sediment. They concluded however that in their area the greater survival was not equal to the additional labor and time involved.

By mid-April the Zostera marina plugs as well as the remaining mats had become heavily infested with the mud snail, (Ilyanassa obsoletus). This mud snail requires a hard substrate to attach its egg cases during its spawning season. The overpopulation of the Z. marina turions was so great that the entire surface areas of the leaves were completely covered with the gastropods. On several occasions the snails were removed from the plants by use of suction and numbers of over several hundred per 0.007 m^2 plug were recorded.

During the first week of May when the water temperature reached 20°C most of the snails were absent from the Zostera marina shoots. However it was at this time that Z. marina rapidly began to deteriorate. Few shoots remained in the transplanted mats but the plugs, which had looked quite healthy the week before, appeared chlorotic in both the fertilized and unfertilized treatments. By May 15 the 0.5 m depth transplants had experienced a significant dieoff of leaves and by the end of May all the treatments had died off to such an extent that only a few shoots of Z. marina remained.

Summer 1979 Transplanting Effort

Because of the poor success of the mats transplanted during the spring of 1979 and the much greater amount of man hours required for the technique, only Zostera marina plugs with attached sediment were transplanted during the summer. Figure 3 presents the percent survival of the plugs at the four transplanted sites along the York River, including the two different depths at the Mumfort Island locations.

The Guinea Marsh site can be considered as a control for the others since it consists of a small unvegetated area surrounded by a very extensive meadow of Zostera marina that archival photography reveals little changed since 1937. It is bordered to the north by a string of low marsh islands dominated by Spartina alterniflora and is located adjacent to the Mobjack Bay region of the Chesapeake Bay. This area has experienced little decline of vegetation in recent years. Comparative biomass data are presented in Section 1 of this report.

The Guinea Marsh site, in contrast to the other transplanted areas, was characterized by less turbidity (secchi >1.0 m) during most periods. This appeared due in part to the baffling effect of the surrounding Zostera marina bed as well as its location in close proximity to the clearer Bay waters. Qualitatively, water clarity within the bed was particularly good during low tidal periods when the baffling effect of the grasses had its greatest impact. The other unvegetated transplant areas appeared much more susceptible during the summer months to resuspension of bottom sediments by wave action, especially during low tides. This baffling effect of the vegetation has been similarly observed by Boynton (personal communication) in Ruppia maritima and Potomageton perfoliatus beds in the upper portion of the Chesapeake Bay.

Survival of the Zostera marina plugs was significantly greater at the Guinea Marsh site than any of the other transplanted areas. Excellent survival of the plugs was recorded in the unfertilized treatment while a significant decrease in survival was observed in the fertilized treatment.

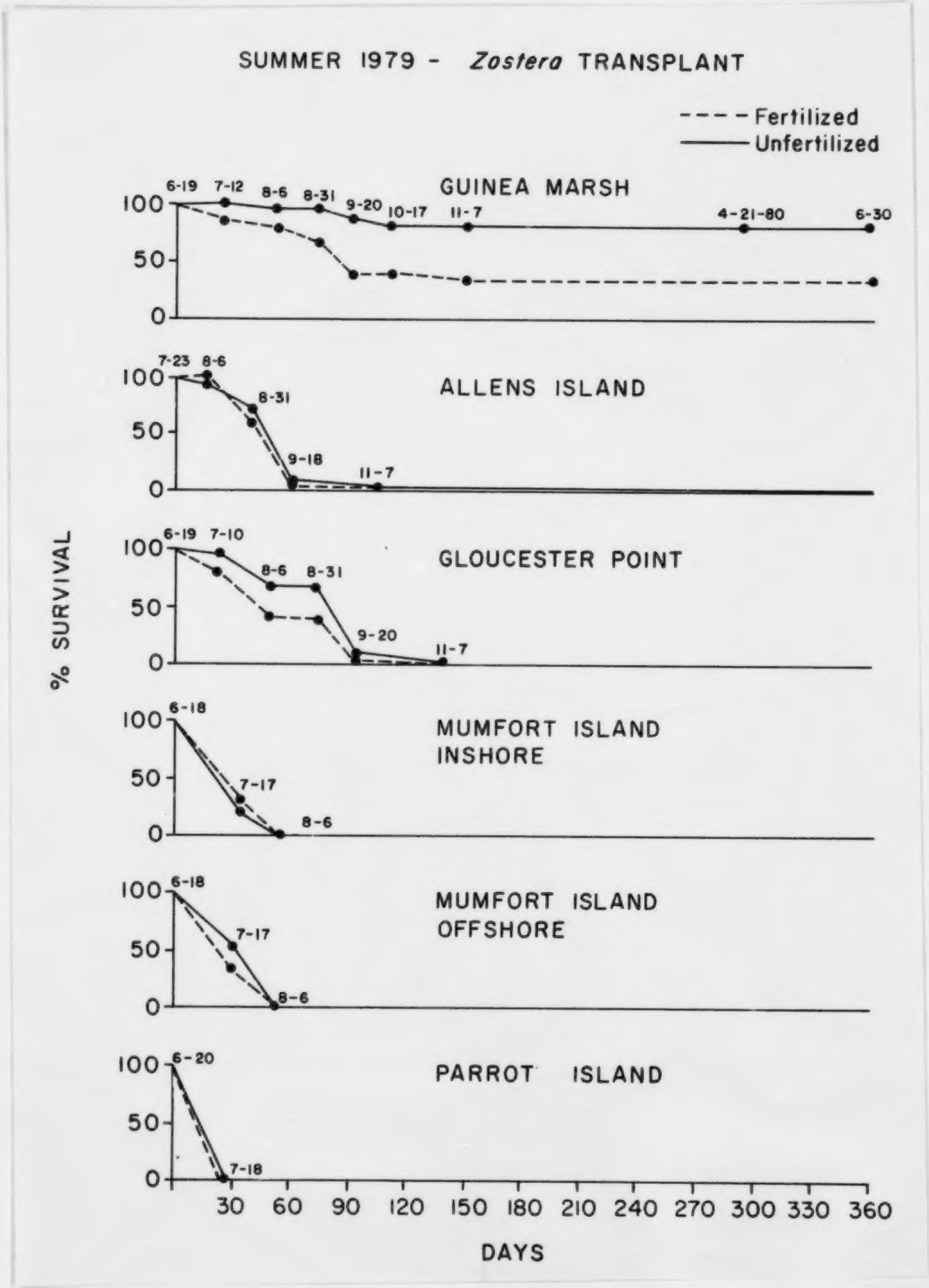

Fig. 3. Percent survival of the Summer, 1979, Zostera marina transplants at the Guinea Marsh, Allen's Island, Gloucester Point, Mumfort Island and Parrot Island Sites.

Table 2 presents the percent survival data for this site on tabular form as well as data on the mean length of the shoots and the mean number of shoots per plug for the study period. Initial losses of plugs during June and July appeared to be the result of uprooting by the physical activity of burrowing organisms, especially the blue crab. The number of shoots per plug remained relatively constant during this period, however the mean length of the shoots rapidly decreased as the tips of the leaves on the longest shoots were broken off by wave action. Little new growth was evident, including the fertilized treatment.

Annual late summer senescence characterized the adjacent vegetation in the SAV bed during months of August and early September and similarly the transplanted plugs showed little new growth during this period. Although the mean length of the shoots remained relatively constant the mean mumber of shoots per plug and the percent survival decreased, especially in the fertilized treatment. The difference between survival in the treatments may have been due more to burrowing by organisms in the fertilized plot rather than an effect of the fertilizer, since blue crabs were observed in holes dug under several remaining plugs, partially dislodging them from the bottom. By late Spetember the apparently stressful period had passed and there was little further loss of plugs. In addition some new growth of vegetation was evident. This compares with a similar period of regrowth observed in the adjacent *Zostera marina* bed (Chapter 1).

Due to vandalism and loss of the marking stakes at the original trasplant site along a section of the river, new *Z. marina* plugs were transplanted at the Allens Island site in July 1979. Scattered patches of *Z. marina* are found in the vicinity. However, the extensive beds of vegetation, many hectares in size, which characterized this area prior to 1973 are gone (Orth, 1976). The summertime turbidity of the water was much higher (0.6-0.8 m, secchi) than that observed for the Guinea Marsh area. As with the other upriver sites it appeared that the extensive surrounding unvegetated flats were susceptible to both waves and tidal currents with considerable resuspension of bottom sediments. This resulted in extremely turbid conditions during many days.

There was a steady loss of the transplants at Allens Island from July through September 1979 (Figure 3). We suspect the poorly developing plants were simply uprooted during periods of high wave energy. Table 3 illustrates the almost immediate decrease in the mean length of the shoots as the longer leaves were broken off by wave action. During August the number of shoots per surviving plug as well as the number of surviving plugs rapidly deceased until by September when there was little left of the original transplants. It is suggested the shock of transplantation in July, combined with the stressful summertime conditions of high temperature, heavy epiphyte growth, and high turbidity precluded the successful establishment of the new vegetation at this site.

Established small patches of vegetation in the vicinity of Allens Island although subject to typical senescence, generally survived the summer. This suggests that although conditions here were more stressful than at Guinea Marsh they would not necessarily preclude the survival of an established bed

TABLE 2. PERCENT SURVIVAL, MEAN LENGTH AND NUMBER OF SHOOTS PER SURVIVING PLUG FOR SUMMER, 1979 ZOSTERA MARINA TRANSPLANT EFFORT AT GUINEA MARSH

Date	Treatment	No. Plugs	% Survival	$\bar{X}$ Length ± s.d. Shoots (cm)	$\bar{X}$ Shoots ± s.d. Plugs
6-19-79	Fertilized	21	100	20 ± 10	10 ± 4
"	Unfertilized	21	100	20 ± 10	10 ± 4
7-12-79	Fertilized	18	86	14 ± 6	9 ± 3
"	Unfertilized	21	100	14 ± 7	12 ± 5
8-6-79	Fertilized	17	81	10 ± 3	5 ± 2
"	Unfertilized	21	100	10 ± 3	9 ± 5
8-31-79	Fertilized	14	67	7 ± 2	4 ± 1
"	Unfertilized	20	95	9 ± 2	7 ± 3
9-20-79	Fertilized	8	38	8 ± 3	2 ± 1
"	Unfertilized	18	86	10 ± 3	5 ± 3
10-17-79	Fertilized	8	38	9 ± 2	2 ±<0.5
"	Unfertilized	17	81	11 ± 2	5 ± 1
11-7-79	Fertilized	7	33	12 ± 3	2 ± 1
"	Unfertilized	17	81	12 ± 4	6 ± 3

TABLE 3. PERCENT SURVIVAL, MEAN LENGTH AND NUMBER OF SHOOTS PER SURVIVING PLUGS FOR SUMMER, 1979 *ZOSTERA MARINA* TRANSPLANT EFFORT AT ALLENS ISLAND SITE.

Date	Treatment	No. Plugs	% Survival	$\bar{X}$ Length±s.d. Shoots (cm)	$\bar{X}$ Shoots±s.d. Plugs
7-23-79	Fertilized	42	100	21 ± 11	8 ± 2
"	Unfertilized	42	100	21 ± 11	8 ± 2
8-6-79	Fertilized	42	100	10 ± 7	5 ± 3
"	Unfertilized	41	98	8 ± 7	8 + 4
8-31-79	Fertilized	25	60	5 ± 2	3 ± 1
"	Unfertilized	29	69	7 ± 2	2 ± 2
9-18-79	Fertilized	2	5	5 ± 2	2 ± 0
"	Unfertilized	3	7	6 ± 3	3 ± 1
11-17-79	Fertilized	0	0	- -	- -
"	Unfertilized	0	0	- -	- -

of SAV. Transplantation during a less stressful time of year than the summer may allow the vegetation to become sufficiently established to survive the critical August conditions.

The Gloucester Point transplant site, in contast to the Guinea Marsh and Allens Island areas, currently is completely devoid of vegetation. It, like all of the other transplanted areas, did contain extensive beds of Zostera marina prior to 1973. Turbidity throughout the stressful late summer months appeared similar to the Allens Island site with secchi disk readings of 0.6 to 0.8 m commonly found.

The transplants showed a steady decline in survival from June with no transplants surviving by November. As with the Guinea Marsh area there seemed to be a slight decrease in the survival of the fertilized versus the unfertilized treatments. The number of shoots per plug rapidly decreased (Table 4) so that by the end of August the remaining plugs consisted of only 2 or 4 small Zostera marina shoots. Likewise there was a rapid decline in the mean length of the shoots as the largest and oldest leaves were removed by wave action with little new vegetative growth to replace them. A small spurt of growth was observed in September, similar to that observed at Guinea Marsh and typical of the growth patterns observed for naturally occurring vegetation in the region. By November however, all the transplanted plugs were gone. We believe that the loss of vegetation during the period of September to November, both at this and the Allens Island site, was not due to the continued deterioraton of the plants, but rather to one of a series of storms occurring during this time.

The Mumfort Island site, the most upstream of all the transplanted areas along the York River, experienced the most rapid dieoff of vegetation with no survival after 50 days (Figure 3). Turbidity always seems highest with secchi disk readings of 0.6 m or less common during the summer. The transplanted Zostera marina exhibited no new growth. Within one month, 75 percent of the transplants had died. There was no apparent difference between the fertilized and unfertilized treatments and at the two depths. By July the mean lengths of the surviving leaves were greatly reduced in length (Table 5) as they rapidly turned brown beginning at their tips and then were broken off by wave action. The tremendous decline of transplants at this site appeared a month or more earlier than that of the downriver areas, suggesting much earlier limiting conditions here.

The Parrot Island transplant site, located along the Rappahannock River, proved quite similar to the Mumfort Island site on the York River. Although documented by aerial photography as having extensive beds of submerged vegetation until the early 1970's, the summer 1979, transplants of Z. marina rapidly declined in abundance. By 50 days after transplantation all the plugs both fertilized and unfertilized had failed (Table 6).

Fall 1979 Transplanting Effort

Initial survival of the plugs of Zostera marina transplanted during September and October 1979 was in nearly complete contrast to the results obtained for those planted during the summer of 1979. The fall transplants

TABLE 4. PERCENT SURVIVAL, MEAN LENGTH AND NUMBER OF SHOOTS PER SURVIVING PLUGS FOR SUMMER, 1979 *ZOSTERA MARINA* TRANSPLANT EFFORT AT THE GLOUCESTER POINT SITES.

Date	Treatment	No. Plugs	% Survival	$\bar{X}$ Length±s.d. Shoots (cm)	$\bar{X}$ Shoots±s.d. Plugs
6-19-79	Fertilized	42	100	20 ± 10	10 ± 4
"	Unfertilized	42	100	20 ± 10	10 ± 4
7-10-79	Fertilized	34	81	13 ± 5	5 ± 4
"	Unfertilized	40	95	14 ± 6	10 ± 6
8-6-79	Fertilized	17	40	11 ± 4	4 ± 3
"	Unfertilized	28	67	11 ± 4	7 ± 2
8-31-79	Fertilized	15	36	5 ± 2	3 ± 1
"	Unfertilized	28	67	7 ± 2	2 ± 2
9-18-79	Fertilized	2	5	13 ± 4	2 ± 1
"	Unfertilized	5	12	8 ± 2	3 ± 1
11-7-79	Fertilized	0	0	- -	- -
"	Unfertilized	0	0	- -	- -

TABLE 5. PERCENT SURVIVAL, MEAN LENGTH AND NUMBER OF SHOOTS PER SURVIVING PLUG FOR SUMMER, 1979 ZOSTERA MARINA TRANSPLANT EFFORT AT THE MUMFORT ISLAND SITES.

Date	Treatment	No. Plugs	% Survival	$\bar{X}$ Length±s.d. Shoots (cm)	$\bar{X}$ Shoots±s.d. Plugs
6-14-79	Fertilized	42	100	22 ± 10	9 ± 5
"	Unfertilized	42	100	22 ± 10	9 ± 5
7-17-79	Fertilized	11	26	6 ± 3	3 ± 2
"	Unfertilized	9	21	7 ± 4	4 ± 2
8-6-79	Fertilized	0	0	- -	- -
"	Unfertilized	0	0	- -	- -
6-18-79	Fertilized	42	100	24 ± 14	6 ± 3
"	Unfertilized	42	100	24 ± 14	6 ± 3
7-17-79	Fertilized	13	31	8 ± 4	4 ± 3
"	Unfertilized	23	55	7 ± 4	5 ± 2
8-6-79	Fertilized	0	0	- -	- -
"	Unfertilized	0	0	- -	- -

TABLE 6. PERCENT SURVIVAL, MEAN LENGTH AND NUMBER OF SHOOTS PER SURVIVING PLUG FOR SUMMER, 1979 ZOSTERA MARINA TRANSPLANT EFFORT AT THE PARROT ISLAND SITE.

Date	Treatment	No. Plugs	% Survival	$\bar{X}$ Length±s.d. Shoots (cm)	$\bar{X}$ Shoots±s.d. Plugs
6-20-79	Unfertilized	84	100	18 ± 8	10 ± 3
6-25-79	Fertilized	42	100	20 ± 11	11 ± 4
7-18-79	Unfertilized	0	0	- -	- -
7-18-79	Fertilized	0	0	- -	- -

at all five of the sites exhibited few losses for at least 180 days (Fig. 4). By the summer of 1980 however, the decline of vegetation experienced in 1979 was again evident. However, this time only the Parrot Island and Mumfort Island sites were severely affected. The Parrot Island losses began between May and June 1980, while the Mumfort Island losses began between July and August 1980, ten months after they were transplanted. The complete loss of all transplanted material at Parrot Island by August 1980, with very little before May, suggests that conditions are quite limiting for the survival of vegetation in that area during these summer months. The decline of vegetation at the Mumfort Island site beginning approximately one month later than Parrot Island suggest that conditions there remain favorable for surivial somewhat longer into the summer. Salinity samples were usually 2 ppt less at Parrot Island than Mumfort Island.

A hypotheis of less stress and increased survival with increasing proximity to the mouth of the rivers is supported by the increased survival evident at the Gloucester Point (VIMS) site located downriver from the Mumfort Island area along the York River. In addition, the nearly 100 percent survival of the transplants at the further downriver Allens Island and Guinea Marsh sites indicates that established beds of vegetation should survive at these areas. This is in fact what is occurring as the Allens Island site approximates the current most upstream limits of naturally occuring Zostera marina. The amounts of vegetation are, however, still greatly reduced from former levels. Recruitment and spreading by seedlings in the fall and winter months from adjacent Z. marina beds may be responsible for many small patches of vegetation found here.

Growth in the transplants as measured by changes in mean area of the plugs and mean number of shoots per plug are presented in Table 7. Some above-ground growth was evident from the September-October transplanting period through December 1979 at all the sites. The plants appeared quite healthy with little of the deterioration observed during the summer. There was no observable effect of the ammonium nitrate fertilizer on the survival or growth of the plugs.

Environmental conditions during this fall period were characterized by decreasing water temperatures (20°C to 5°C) and reduced turbidity at all sites. During August 1979, secchi disk readings varied from approximately 0.6 m at the most upstream sites, Mumfort and Parrot Islands, to 1.0 m at Guinea Marsh. From October through December however, it appeared that all sites had secchi disk readings of 1.0 m or greater.

The period of December 1979 to June 1980 was characterized by tremendous growth of the fall transplanted vegetation at all of the sites. The Allens Island site showed the greatest increase with 17 and 20 fold increases in mean plug area between December and May for the September 1979 for fertilized and unfertilized transplants, respectively, and 14 and 15 fold increases in areas for the October 1979, fertilized and unfertilized transplants. Increases in the numbers of shoots were 12 and 14 fold and 6 and 11 fold, respectively. By June 1980, all of the transplants in the various treatments at this site had grown together so that observations of individual plugs became impossible. This period of active growth parallels that observed for

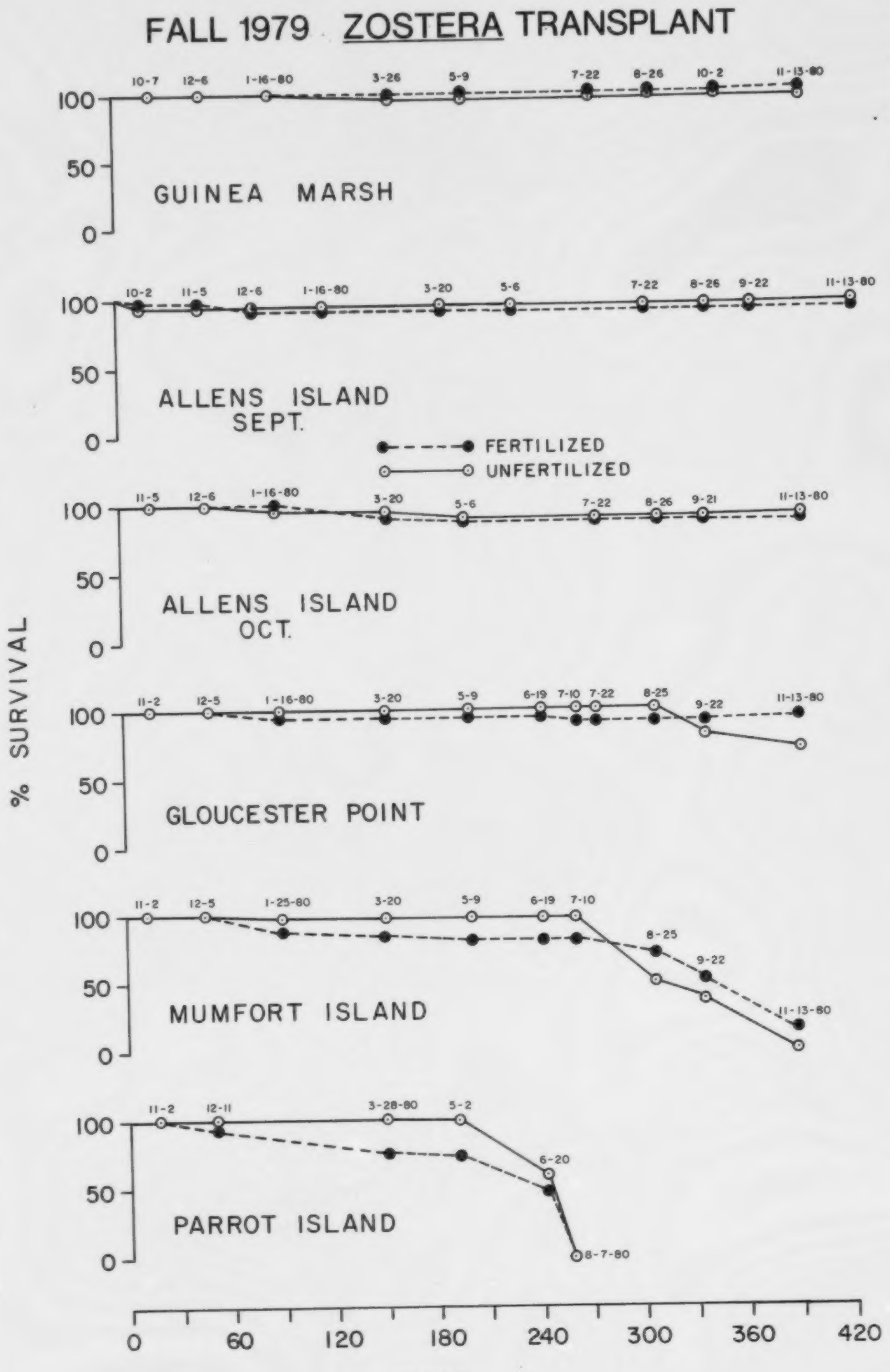

Fig. 4. Percent survival of Fall, 1979, *Zostera marina* transplants.

TABLE 7. MEAN AREA OF PLUGS (cm^2) AND MEAN NUMBER OF ZOSTERA MARINA SHOOTS PER PLUG FOR FALL, 1979 TRANSPLANTS.

Location		Area	No. Shoots	Area	No. Shoots	Area	No. Shoots	Area	No. Shoots	Area	No. Shoots
	Date			10-19-79		11-7-79		12-6-79		5-9-80	
Guinea Marsh	fertilized			69	12	69	13	69	12	437	37
	unfertilized			69	12	69	9	69	14	262	43
	Date	9-19-79		10-2-79		11-5-79		12-6-79		5-6-80	
Allens Is. (Sept.)	fertilized	69	9	69	9	69	13	69	11	1404	150
	unfertilized	69	9	69	8	69	9	69	14	1202	166
	Date			10-19-79		11-5-79		12-6-79		5-6-80	
Allens Is. (Oct.)	fertilized			69	12	69	11	69	13	1048	147
	unfertilized			69	12	69	12	69	16	937	99
	Date			10-19-79		11-2-79		12-5-79		5-9-80	
Gloucester Pt.	fertilized			69	11	69	13	69	17	**	**
	unfertilized			69	11	69	13	69	13	**	**
	Date			10-19-79		11-2-79		12-5-79		5-9-80	
Mumfort Is.	fertilized			69	14	69	14	69	15	**	**
	unfertilized			69	14	69	13	69	14	**	**
	Date			10-19-79		11-9-79		12-11-79		5-2-80	
Parrot Is.	fertilized			69	12	69	12	69	16	824	121
	unfertilized			69	12	69	14	69	17	884	113

- no data
* no data individual plugs grown together
** no data mud snail infestation

TABLE 7. CONTINUED

Location		Area	No. Shoots	Area	No. Shoots	Area	No. Shoots	Area	No. Shoots	Area	No. Shoots
	Date	7-22-80		8-26-80		10-2-80		11-13-80			
Guinea Marsh	fertilized	1790	200	*	*	*	*	*	*		
	unfertilized	1417	153	*	*	*	*	*	*		
	Date	6-19-80		7-24-80		8-26-80		9-22-80		11-13-80	
Allens Is. (Sept.)	fertilized	*	*	*	*	*	*	*	*	*	*
	unfertilized	*	*	*	*	*	*	*	*	*	*
	Date	6-19-80		7-24-80		8-26-80		9-22-80		11-13-80	
Allens Is. (Oct.)	fertilized	*	*	*	*	*	*	*	*	*	*
	unfertilized	*	*	*	*	*	*	*	*	*	*
	Date	6-19-80		7-22-80		8-25-80		9-22-80		11-13-80	
Gloucester Pt.	fertilized	644	99	1021	111	754	131	-	70	1430	118
	unfertilized	739	95	1080	114	942	86	-	30	699	50
	Date	6-19-80		7-24-80		8-25-80		9-22-80		11-13-80	
Mumfort Is.	fertilized	**	**	1338	130	742	36	-	16	246	17
	unfertilized	**	**	1711	166	424	23	-	3	0	0
	Date	6-20-80		8-7-80							
Parrot Is.	fertilized	989	135	0	0						
	unfertilized	985	123	0	0						

- no data
* no data individual plugs grown together
** no data mud snail infestation

existing beds of Zostera marina found in this region of the lower Chesapeake Bay (Chapter 1). The slightly better growth of the September transplants as compared to the October transplants at the Allens Island site suggests that an additional period of growth during the fall is initially beneficial to the re-establishment of vegetation in this area. However, the steady decline of all of the transplanted plugs placed at this same location on July 23, 1979 suggests a significant change had occurred between July and September in environmental factors which had previously been limiting the establishment of new vegetation.

A heavy infestation of mud snails (Ilyanassa obsoletus) was evident from April to June 1980, at the Gloucester Point and Mumfort Island sites along the York River. As described previously, a similar infestation was observed at Mumfort Island in April 1979. Their presence in extreme numbers may be due to the lack of vegetation in these areas so that there is little suitable substrate for laying their eggs. Although the transplanted plugs were impacted to such a degree that hundreds of the snails completely smothered the plants for weeks at a time, the vegetation recovered and continued growing at both sites until August 1980.

From August to September 1980, the characteristic late summer senescence occurred at all the York River sites. The Guinea Marsh and Allens Island areas had become so well established that they were not critically affected by this period and new growth was evident after September. The Gloucester Point site showed a considerable decline in the numbers of shoots between August and September but considerable regrowth was evident by November. The upriver Mumfort Island site again showed the greatest decline along the York River areas in August with little surviving vegetation by November.

The Parrot Island site, located along the Rappahannock River, showed its characteristic earlier and more severe decline than any of the York River areas. Growth was observed throughout the spring until the June 20 sampling but between this date and August 7, there was a precipitous decline with all vegetation gone by this latter date. It appears evident, therefore, that revegetation of this section of the Rappahannock is limited by environmental conditions present during July.

Spring 1980 Transplanting Effort

Particle size distribution of the sediments within the 0-2, 2-5, 5-10 and 10-15 cm depth segments of the cores are presented in Table 8 for the four unvegetated York River transplant sites as well as the Guinea Marsh donor site. The statistical parameters of grain size calculated for these data are presented in Table 9. These sediment cores were taken on March 14, 1980, several weeks prior to the Spring 1980 transplantation of Zostera marina at these areas.

Analysis of the particle size distribution indicated that the sediments within each site were quite homogeneous with respect to depths of at least 15 cm. The graphic mean (M_2) and median (Md) measures of average size showed little change with depth within each core. The inclusive standard deviation

TABLE 8. PARTICLE SIZE DISTRIBUTION (%) FOR DEPTH INTERVALS OF SEDIMENT CORES AT TRANSPLANT AND DONOR SITES ALONG THE YORK RIVER, 3-14-80.

		Depth	mm	1.000	.500	.250	.125	.063	<.063
Location	Core#	(cm)	Ø	0	1	2	3	4	5
Mumfort Is.	1	0-2		0.22	5.50	60.20	25.50	1.23	6.98
"	"	2-5		0.13	6.70	61.90	21.20	1.25	7.91
"	"	5-10		0.42	3.48	57.30	27.10	1.68	9.11
"	"	10-15		0.23	3.40	63.50	22.20	1.62	8.76
Gloucester Pt.	1	0-2		1.04	4.24	52.13	29.71	1.92	10.14
"	"	2-5		0.19	1.24	53.81	34.76	2.03	7.51
"	"	5-10		0.44	1.49	50.03	37.20	1.88	6.98
"	"	10-15		0.82	2.15	53.46	32.76	2.08	7.95
Allens Is.	1	0-2		0.66	1.53	27.95	54.60	5.73	9.08
"	"	2-5		0.18	1.41	26.50	54.78	9.02	8.86
"	"	5-10		0.59	1.43	28.82	54.26	5.80	8.49
"	"	10-15		0.66	1.68	35.63	46.78	7.64	8.69
Guinea Marsh (unvegetated)	1	0-2		0.55	0.61	13.49	64.86	9.58	10.14
"	"	2-5		0.13	0.26	3.25	78.19	7.96	7.51
"	"	5-10		0.20	0.46	3.45	75.28	9.87	6.98
"	"	10-15		0.39	0.55	7.52	75.94	5.72	7.95
Guinea Marsh (vegetated)	1	0-2		2.49	1.64	9.22	56.70	14.64	13.63
"	"	2-5		0.50	0.87	14.87	59.04	10.57	12.86
"	"	5-10		1.01	0.76	12.30	63.85	9.00	11.18
"	"	10-15		0.90	0.82	8.12	70.03	8.88	10.71

TABLE 9. STATISTICAL PARAMETERS OF GRAIN SIZE FOR DEPTH INTERVALS OF SEDIMENT CORES AT TRANSPLANT AND DONOR SITES ALONG THE YORK RIVER, 3-14-80.

Location	Core #	Depth (cm)	Mean (M)	Median (Md)	Sorting (σI)	Skewness (SK_1)
Mumfort Is.	1	0-2	1.9	1.8	0.81	+0.33
"	"	2-5	1.9	1.7	0.86	+0.42
"	"	5-10	2.0	1.8	0.81	+0.43
"	"	10-15	2.0	1.8	0.79	+0.46
Gloucester Pt.	1	0-2	2.0	1.9	0.85	+0.35
"	"	2-5	2.1	2.0	0.74	+0.45
"	"	5-10	2.1	2.0	0.77	+0.34
"	"	10-15	2.0	1.9	0.80	+0.37
Allens Is.	1	0-2	2.3	2.3	0.77	+0.15
"	"	2-5	2.4	2.4	0.80	+0.14
"	"	5-10	2.3	2.3	0.77	+0.15
"	"	10-15	2.3	2.2	0.78	+0.22
Guinea Marsh (unvegetated)	1	0-2	2.7	2.6	0.75	+0.21
"	"	2-5	2.7	2.6	0.55	+0.34
"	"	5-10	2.8	2.6	0.61	+0.34
"	"	10-15	2.6	2.6	0.58	+0.18
Guinea Marsh (vegetated)	1	0-2	2.4	2.6	0.94	+0.18
"	"	2-5	2.8	2.6	0.88	+0.28
"	"	5-10	2.8	2.6	0.84	+0.28
"	"	10-15	2.7	2.6	0.70	+0.25

or sorting coefficient (σI) indicated that the sediments were moderately sorted (Folk, 1968) at all depths at all sites. The inclusive graphic skewness measure (SK_1) revealed the sediments to be fine-skewed to strongly fine-skewed with little effect of depth. This homogeneity of the sediments within each core were similar to the results obtained at a *Zostera marina* and *Ruppia maritima* bed located nearby at Brown's Bay and presented in Section 5 of this report.

Between-site variation was significantly greater than within site variation with depth. The most upriver Mumfort Island site had the largest (smallest phi) median and mean measures of average size (Table 9). Proceeding downriver, each transplant site had an incremental reduction in the average size of the sediment particles with the finest sediments found at the Guinea Marsh area located at the mouth of the river. Analysis of the particle size distribution information (Table 8) reveals a shift from 1 and 2 phi particles at Mumfort Island to 3 and 4 phi at Guinea Marsh site with intermediate values at Gloucester Point and Allens Island areas. This gradation in size may be representative of large scale sorting of littoral sediments from upriver to downriver or simply may be an artifact of more localized physical sedimentation processes such as distance from an adjacent sediment source. Within the Guinea Marsh area the vegetated core had the largest percentages of fine material (4 and 5 phi particles) as might be expected. Although there were differences in particle sizes between transplant sites these slight differences are certainly within the range of sediments where *Zostera marina* is found locally and would not preclude the reestablishment of vegetation. The relatively small difference between the unvegetated, denuded areas and the vegetated bed as well as data from earlier studies in the region (Orth, 1973) suggests that there has been little appreciable change in the sediment type along the York River since the disappearance of the *Z. marina* beds.

Extractable sediment pore water and surface water nutrient concentrations for replicate cores taken at the Guinea Marsh, Allens Island, Gloucester Point and Mumfort Island transplant sites on March 14, 1980 are presented in Tables 10,11, 12 and 13, respectively. Similar data were obtained for cores taken in a *Zostera marina* and *Ruppia maritima* bed at Brown's Bay and are presented in Section 5 of this report.

Ammonium levels in the sediments at each of the transplant sites show little significant variation between sites. There were few obvious patterns of change with depth, however several of the cores exhibited lowest concentrations at depths less than 2 cm. This may be the result of the diffusion of mineralized ammonium from the sediment into the water column or aerobic nitrification. Higher ammonium levels in the 0-2 cm layer of the vegetated versus unvegetated Guinea Marsh cores is similar to that observed in the vegetated and unvegetated cores at Brown's Bay (Section 5). This may be due to the greater perturbation of the sediments within the unvegetated area resulting in increased diffusion or denitrification of ammonium when compared to the more protected vegetated zone or less detrital input.

Nitrate levels in the sediments were extremely low at the Mumfort Island and Gloucester Point transplant sites suggesting rapid uptake or

TABLE 10. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (µM) AT GUINEA MARSH TRANSPLANT AND DONOR SITES, 3-14-80.

Core	Depth	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
Vegetated-1	water	0.8	1.0	0.1	0.4
"	0-2	92.1	6.91	1.53	7.84
"	2-5	78.8	8.16	1.30	21.6
"	5-10	56.0	0.91	2.24	16.1
"	10-15	37.4	0.28	0.91	8.11
Vegetated-2	water	0.6	0.4	0.2	0.6
"	0-2	107	3.03	3.52	18.0
"	2-5	209	2.04	3.86	50.2
"	5-10	44.8	0.57	1.36	12.4
"	10-15	50.4	0.57	0.99	8.40
Unvegetated-1	water	0.4	0.6	0.1	0.3
"	0-2	25.6	1.43	2.27	8.85
"	2-5	96.7	0.88	2.95	27.2
"	5-10	170	2.94	1.50	17.2
"	10-15	206	0.32	0.96	11.6
Unvegetated-2	water	0.4	0.5	0.96	0.3
"	0-2	10.9	1.03	1.64	8.15
"	2-5	46.9	1.11	1.50	16.8
"	5-10	79.1	0.45	1.11	13.8
"	10-15	97.9	1.19	1.08	11.5

TABLE 11. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (μM) AT ALLENS ISLAND TRANSPLANT SITE, 3-14-80.

Core	Depth	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
Unvegetated 1	water	0.6	0.1	0.1	0.3
"	0-2	55.1	<0.01	9.04	9.76
"	2-5	172	<0.01	6.55	46.8
"	5-10	90.7	2.82	11.4	79.6
"	10-15	119	0.81	1.56	38.6
Unvegetated 2	water	0.4	0.2	0.1	0.4
"	0-2	82.9	<0.01	1.08	11.48
"	2-5	25.0	0.27	0.68	4.66
"	5-10	78.0	0.05	0.62	7.62
"	10-15	92.0	7.79	2.38	8.43

TABLE 12. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (μM) AT GLOUCESTER POINT TRANSPLANT SITE, 3-14-80.

Core	Depth	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
Unvegetated 1	water	0.56	1.05	0.14	0.36
"	0-2	20.0	<0.01	1.68	7.07
"	2-5	28.3	<0.01	1.98	15.0
"	5-10	7.28	<0.01	0.47	10.1
"	10-15	103	<0.01	0.58	14.3
Unvegetated 2	water	0.48	0.72	0.14	0.37
"	0-2	9.33	<0.01	0.47	3.14
"	2-5	45.9	<0.01	1.76	17.8
"	5-10	103	<0.01	5.31	25.3
"	10-15	162	<0.01	6.63	31.3

TABLE 13. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (μM) AT MUMFORT ISLAND TRANSPLANT SITE, 3-14-80.

Core	Depth	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
Unvegetated 1	water	0.07	3.95	0.16	0.27
"	0-2	16.4	<0.01	0.30	0.97
"	2-5	73.1	<0.01	0.36	3.61
"	5-10	116	<0.01	0.50	4.86
"	10-15	86.2	<0.01	0.33	2.43
Unvegetated 2	water	0.83	3.72	0.15	0.25
"	0-2	12.7	<0.01	0.50	2.99
"	2-5	43.3	<0.01	2.06	18.2
"	5-10	78.6	<0.01	0.85	17.8
"	10-15	73.1	<0.01	0.52	15.1

denitrification by bacteria of any available nitrate with little accumulation. The Allens Island site showed somewhat higher levels in the sediments. Interestingly the highest levels were recorded in the Guinea Marsh vegetated cores at depths less than 5 cm.

Nitrite levels were consistently higher than nitrate levels in the sediments at all sites except for the Guinea marsh area. Conversely nitrite levels in the water were consistently lower than nitrate.

Inorganic phosphate levels varied considerably but were generally comparable at the vegetated donor site and the unvegetated transplant areas. Similar values were obtained for vegetated and unvegetated areas in the Brown's Bay region (Chapter 5). Within each unvegetated core lowest levels of phosphate generally occurred in the top 0-2 cm of sediment.

Table 14 presents the results for the extractable sediment pore water and surface water nutrient concentrations for the unfertilized, vegetated Zostera marina transplants at the Allens Island site at 10 days (4-17-80) and 37 days (5-14-80) after transplantation. Table 15 presents results of similar data for the existing unvegetated and unfertilized sediments at the site. In general there appears little difference between the vegetated and unvegetated cores at each of the dates. Phosphate levels are higher in the vegetated, May 14, samples as compared to the unvegetated cores however extractable phosphate levels varied considerably.

Both treatments show similar patterns for several of the nutrient species. Nitrate levels were considerably higher in both the vegetated and unvegetated cores during the April 17, sampling than on May 14. All other nutrient levels were comparable during both dates. Ammonium and inorganic phosphate levels were generally lowest in the 0-2 cm sections of the cores on both dates in both treatments. Possibly uptake, conversion or loss of these two species into the water column through diffusion is occurring. Regardless of their fate there was little significant effect of the vegetation evident on the extractable nutrient concentration in the sediments.

Levels of extractable nutrients in the unvegetated sediments 10 and 37 days after treatments with Osmocote or ammonium nitrate fertilizers are presented in Tables 16 and 17, respectively. Depths of fertilizer placement varied between 10 to 15 cm below the surface. Both fertilizers showed tremendous increases in all the nitrogen species. Due to the anaerobic conditions, and the types of fertilizer used, the largest fraction of nitrogen was present as ammonium, a species that is chemically stable under reducing conditions. Highest concentrations of ammonium were found at depths of 10 to 15 cm with a gradient of concentration to the sediment surface in these unvegetated areas. High levels of ammonium in the overlying water 10 days after application indicates initial significant losses by diffusion into the water column. Continued high levels of ammonium were found in the sediments 37 days after transplantation in both treatments. The Osmocote treatment, due to the slow release nature of the fertilizer, would be expected to continue these higher levels of ammonium for a considerably longer period than ammonium nitrate.

TABLE 14. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (μM) AT ALLENS ISLAND TRANSPLANT SITE, VEGETATED AND UNFERTILIZED PLUGS.

Date	Core	Depth (cm)	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
4-17-80	#1	water	0.20	<0.01	2.13	2.65
"	"	0-2	20.5	16.4	3.84	7.87
"	"	2-5	64.0	24.0	6.71	18.5
"	"	5-10	125	82.9	3.66	19.0
"	"	10-15	154	66.6	2.01	19.1
4-17-80	#2	water	0.01	<0.01	2.04	3.50
"	"	0-2	35.0	6.66	0.44	0.76
"	"	2-5	121	4.52	1.40	13.2
"	"	5-10	181	5.04	0.61	14.8
"	"	10-15	87.2	24.3	1.05	4.00
5-14-80	#1	water	0.20	0.05	0.11	0.29
"	"	0-2	12.8	0.87	0.78	4.47
"	"	2-5	83.9	1.31	0.87	23.2
"	"	5-10	117.7	0.60	0.96	46.3
"	"	10-15	3.13	0.62	0.61	3.72
5-14-80	#2	water	0.09	0.12	0.10	0.28
"	"	0-2	8.63	0.28	0.78	2.80
"	"	2-5	80.5	0.31	0.96	29.0
"	"	5-10	245	<0.01	2.79	43.6
"	"	10-15	149	0.09	0.78	3.98

TABLE 15. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (µM) AT ALLENS ISLAND TRANSPLANT SITE, UNVEGETATED AND UNFERTILIZED PLUGS.

Date	Core	Depth (cm)	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
4-17-80	#1	water	<0.01	<0.01	2.13	2.89
"	"	0-2	14.6	6.07	0.35	0.99
"	"	2-5	150	49.1	0.70	2.18
"	"	5-10	52.2	3.31	0.96	11.0
"	"	10-15	126	37.2	4.10	10.6
4-17-80	#2	water	<0.01	<0.01	2.30	2.84
"	"	0-2	8.68	9.85	2.01	4.27
"	"	2-5	233	185	9.68	18.7
"	"	5-10	201	52.6	9.94	39.2
"	"	10-15	144	6.86	1.05	15.2
5-14-80	#1	water	0.02	0.06	0.10	0.24
"	"	0-2	6.36	0.66	0.61	0.60
"	"	2-5	30.4	0.06	0.78	3.86
"	"	5-10	89.1	<0.01	0.61	9.62
"	"	10-15	101	0.30	0.61	5.44
5-14-80	#2	water	<0.01	0.02	0.08	0.19
"	"	0-2	12.4	0.13	0.61	1.13
"	"	2-5	128	0.20	0.61	11.1
"	"	5-10	166	1.72	0.52	13.3
"	"	10-15	97.8	0.12	0.52	7.18

TABLE 16. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (μM) AT ALLENS ISLAND TRANSPLANT SITE, UNVEGETATED PLUGS FERTILIZED WITH OSMOCOTE.

Date	Core	Depth (cm)	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
4-24-80	#1	water	12.0	7.77	0.23	0.20
"	"	0-2	13400	1540	708	11.0
"	"	2-5	30500	6570	1550	162
"	"	5-10	23000	6840	1620	314
"	"	10-15	23100	7790	130	352
4-24-80	#2	water	41.2	24.9	5.01	0.49
"	"	0-2	13600	4520	120	6.77
"	"	2-5	38200	16400	488	142
"	"	5-10	41700	21700	4290	315
"	"	10-15	78100	51000	444	534
5-21-80	#1	water	1.31	0.03	0.06	0.16
"	"	0-2	4540	450	<0.01	1040
"	"	2-5	10700	131	<0.01	1020
"	"	5-10	55000	861	32.4	819
"	"	10-15	21000	1120	410	510
5-21-80	#2	water	2.16	0.67	0.34	0.22
"	"	0-2	3650	127	3.82	13.0
"	"	2-5	5130	400	112	252
"	"	5-10	18700	7690	11.9	1830
"	"	10-15	27400	14800	305	2090

TABLE 17. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (µM) AT ALLENS ISLAND TRANSPLANT SITE, UNVEGETATED PLUGS FERTILIZED WITH AMMONIUM NITRATE.

Date	Core	Depth (cm)	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
4-24-80	#1	water	0.88	0.07	0.13	0.21
"	"	0-2	5680	683	0.24	2.99
"	"	2-5	15600	9450	9.29	25.4
"	"	5-10	24600	14600	2220	26.6
"	"	10-15	37700	15000	8160	5.97
4-24-80	#2	water	17.2	11.6	5.04	0.20
"	"	0-2	12600	3220	1420	2.12
"	"	2-5	18800	6710	2150	2.07
"	"	5-10	24800	14000	2620	2.05
"	"	10-15	35600	24800	3950	3.19
5-21-80	#1	water	0.12	<0.01	0.06	1.18
"	"	0-2	4130	131	<0.01	35.7
"	"	2-5	6870	3.77	<0.01	72.4
"	"	5-10	14700	438	74.4	61.6
"	"	10-15	21100	4420	1700	18.3
5-21-80	#2	water	0.53	0.16	<0.01	1.87
"	"	0-2	5780	578	8.01	4.31
"	"	2-5	6310	291	3.51	35.8
"	"	5-10	10400	872	4.21	32.1
"	"	10-15	11300	1530	2.48	7.25

Levels of nitrate and nitrite in the interstitial were also raised considerably by the additions of the fertilizers to the unvegetated sediments. Highest levels were again found at depths between 10 and 15 cm with lowest levels in the upper two centimeters near the sediment surface. High levels in the overlying water indicate considerable diffusion into the water column was occurring 10 days after transplantation. Reduced levels of nitrate and nitrite in the sediments found 37 days after application indicate much of these two inorganic nitrogen species had been lost. Most likely uptake, diffusion and denitrification are responsible for these reductions. Highest levels were evident during this period at depths below 10 cm suggesting some continued input of these two oxidized forms of nitrogen from the fertilizers.

Levels of phosphate in the sediments differed between the two fertilized treatments. Since no phosphate was present in the ammonium nitrate fertilizer, levels of inorganic phosphorous were comparable to the unfertilized treatments during these dates. Osmocote on the other hand which was 14 percent phosphate, raised the levels in the sediments considerably, although not nearly as high as for the nitrate and ammonium component. This suggests that much of the phosphorus supplied by the fertilizer was being precipitated with ferric iron or other heavy metals and bound in the sediments.

Tables 18 and 19 present the results of the sediment and water nutrient concentrations for the vegetated plugs fertilized with Osmocote and ammonium nitrate, respectively. On April 17, ten days after transplantation levels of ammonium in both of the osmocote treatment cores and one of the ammonium-nitrate cores were considerably less than that observed in the unvegetated treatments, suggesting uptake of ammonium by the plants was occurring. After 37 days levels of ammonium in the osmocote transplants increased slightly while those in the ammonium nitrate treatment showed varied results. Concentrations in the sediments were highest in both treatments at the 10-15 cm depths with reduced levels towards the surface.

Levels of nitrate and nitrite in the sediments generally showed considerable declines from 10 to 37 days after application in a similar manner to that experienced by the fertilized, unvegetated plugs. High levels of nitrate and nitrite in the surface water at 10 days after application indicates a considerable amount of leaching was initially important. Other reductions in the levels may have been due to denitrification, uptake by the plants and sediment microorganisms.

Phosphate levels showed significant increases over the nonfertilized treatments at 10 and 37 days after application for only the osmocote fertilized plugs. This is similar to the results observed for the unvegetated plugs. Reduced levels of phosphate were observed in several of the cores in the 0-2 cm depth interval in vegetated as well as the unvegetated cores. This suggests either diffusion into the surface water or precipitation of upward diffusing phosphate in an insoluble form at this aerobic layer.

TABLE 18. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (µM) AT ALLENS ISLAND TRANSPLANT SITE, VEGETATED PLUGS FERTILIZED WITH OSMOCOTE.

Date	Core	Depth (cm)	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
4-17-80	#1	water	17.8	3210	3.15	3.63
"	"	0-2	404	47.5	0.94	35.7
"	"	2-5	799	158	1.70	9.45
"	"	5-10	994	9170	12.8	---
"	"	10-15	999	19400	8.42	241
4-17-80	#2	water	3.63	42.4	<0.01	4.65
"	"	0-2	630	53.3	37.8	41.5
"	"	2-5	986	53.1	62.3	302.3
"	"	5-10	997	286	165	511
"	"	10-15	999	63500	68.6	599
5-14-80	#1	water	0.74	<0.01	0.25	0.28
"	"	0-2	7040	6240	2.21	2.85
"	"	2-5	5700	54.8	2.80	107
"	"	5-10	11100	<0.01	297	412
"	"	10-15	21500	3650	516	414
5-14-80	#2	water	0.27	<0.01	0.07	0.48
"	"	0-2	2480	<0.01	0.86	4.23
"	"	2-5	2190	<0.01	1.45	8.22
"	"	5-10	1910	<0.01	1.45	6.41
"	"	10-15	4470	547	4.15	67.8

TABLE 19. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (μM) AT ALLENS ISLAND TRANSPLANT SITE, VEGETATED PLUGS FERTILIZED WITH AMMONIUM NITRATE.

Date	Core	Depth (cm)	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
4-17-80	#1	water	2.76	20.3	7.97	3.11
"	"	0-2	801	598	1.28	38.6
"	"	2-5	995	46100	1.79	73.0
"	"	5-10	996	4660	2.89	59.9
"	"	10-15	995	46100	1.79	73.0
4-17-80	#2	water	22.1	847	491	2.79
"	"	0-2	937	3050	4.42	11.1
"	"	2-5	40100	21000	515	28.7
"	"	5-10	124000	132000	644	98.2
"	"	10-15	9400	139000	386	34.6
5-14-80	#1	water	0.25	0.01	0.05	-
"	"	0-2	4330	322	<0.01	2.67
"	"	2-5	5600	386	<0.01	2.67
"	"	5-10	7390	449	<0.01	4.24
"	"	10-15	6160	614	24.9	5.44
5-14-80	#2	water	0.22	0.03	0.06	0.20
"	"	0-2	3150	195	<0.01	2.96
"	"	2-5	4530	322	<0.01	5.40
"	"	5-10	6740	323	<0.01	2.32
"	"	10-15	7180	321	0.59	0.01

Percent survival of the Zostera marina plugs transplanted in April 1980, at the four York River transplant sites are presented in Figure 5. Few losses were observed at any of the sites until June, 19, after which time the Mumfort Island site began a precipitous decline. Loss of plugs during the summer months at this location parallels the results of every other transplant effort at this site regardless of when initiated. Gloucester Point and Allens Island locations demonstrated intermediate levels of survival with Osmocote fertilized plugs at the Allens Island site having the greatest success. The Guinea Marsh control site in contast to other areas showed no loss of plugs up to the end of the study period in November 1980.

Growth or decline of the plugs as evidenced by mean area of the plugs and mean number of shoots per plug is presented in Table 20. Figure 6 presents graphically the mean number of shoots per plug data. Guinea Marsh transplants demonstrated over a three-fold increase in number of shoots per plug and a fifteen-fold increase in area from April to July. Summertime senescence was evident from July through September while an increase in both area and number of shoots was evident from October to November.

An effect of fertilizers on growth was evident at the Allens Island treatments. All three treatments showed increases in the number of shoots per plug from April to July with growth of the Osmocote fertilizer continuing until August. The greatest response was evident in the Osmocote treatment followed by the ammonium nitrate fertilizer. Although the sediment nutrient analyses showed extremely high levels of ammonia after 37 days for both fertilizers, continued high levels would be expected from Osmocote because of its slow release nature. In addition, although inorganic phosphorus has not been regarded as limiting to growth of submerged grasses, high levels found in the sediments after application of Osmocote indicate that it cannot be ruled out as a contributing factor to the growth in this case. Senescence was evident in all three treatments in late summer from August to September while an additional characteristic spurt of growth was observed from September to November.

Application of Osmocote resulted in a 325 percent increase in the mean number of shoots per plug over the unfertilized treatment and a 220 percent increase in mean area. The ammonium nitrate on the other hand showed only a 40 percent increase in the number of shoots and a 71 percent increase in area. Churchill et al., 1978, found little positive effect of Osmocote on the growth or survival of his miniplugs transplanted in Long Island. His application rates, (3.5 g vs 40 g here), as well as his application techniques, suggest there was limited availability of the fertilizer for uptake by the plants.

The Gloucester Point transplant site was heavily impacted by mud snails during April and May 1980. Effect of the snails on the growth of the plugs is evidenced by a comparison of the caged and uncaged treatments (Fig. 5 and Table 20). The total number of surviving plugs, mean area of the plugs and the mean number of shoots per plug were significantly greater in the caged versus the uncaged treatment on June 16, 1980. At this time the snails had completed their egg laying and were for the most part, gone from the Zostera marina plants. The cages were therefore removed. By July 22, 1980 the

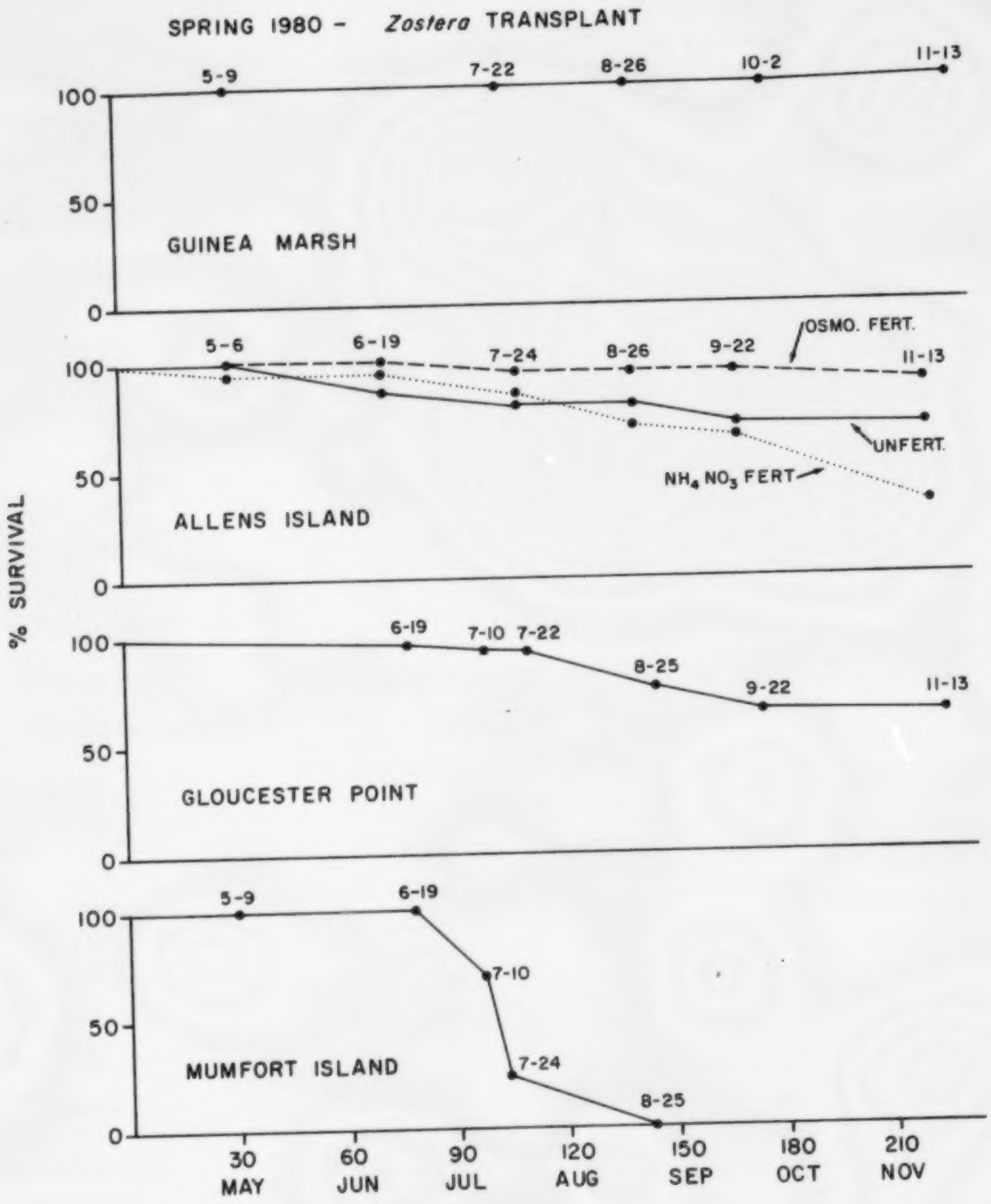

Fig. 5. Percent survival of Spring, 1980, Zostera marina transplants.

TABLE 20. MEAN AREA OF PLUGS (CM^2) AND MEAN NUMBER OF SHOOTS PER PLUG FOR SPRING, 1980, TRANSPLANTS.

Location		Area	No. Shoots	Area	No. Shoots	Area	No. Shoots	Area	No. Shoots
	Date	4-2-80		5-9-80		6-19-80		7-22-80	
Guinea Marsh		69	28	-	28	-	-	1067	94
	Date	4-7-80		5-6-80		6-19-80		7-24-80	
Allens Is.	fertilizer 1	69	14	-	18	1079	141 *	1319 *	197 *
	fertilizer 2	69	14	-	18	1287	102	1077 *	113 *
	unfertilized	69	15	-	18	366 *	64	773 *	71 *
	Date	4-2-80		5-9-80		6-19-80		7-22-80	
Gloucester Pt.	caged	69	15	-	-	612 *	49 *	695 *	85 *
	uncaged	69	15	+	+	174	22	154	17
	Date	4-2-80		5-9-80		6-19-80		7-10-80	
Mumfort Is.		69	15	+	+	+	+	404	28

- no data
+ no data mud snail infestation
fertilizer 1 - Osmocote (14-14-14)
fertilizer 2 - Ammonium nitrate (37-0-0)
* sign. diff. @ 0.05

TABLE 20. CONTINUED

Location		Area	No. Shoots	Area	No. Shoots	Area	No. Shoots
	Date	8-26-80		10-2-80		11-13-80	
Guinea Marsh		848	58	519	33	671	58
	Date	8-26-80		9-22-80		11-13-80	
Allens Is.	fertilizer 1	1680*	271*	-	212*	1901*	221*
	fertilizer 2	840	92	-	63	1221	73
	unfertilized	852	60	-	37	715	52
	Date	8-25-80		9-22-80		11-13-80	
Gloucester Pt.	caged	1114*	70*	-	36*	604*	40*
	uncaged	479	14	-	7	157	13
	Date	7-24-80		8-26-80		11-13-80	
Mumfort Is.		242	16	0	0	0	0

- no data
+ no data mud snail infestation
fertilizer 1 - Osmocote (14-14-14)
fertilizer 2 - Ammonium nitrate (37-0-0)
* sign. diff. @ 0.05

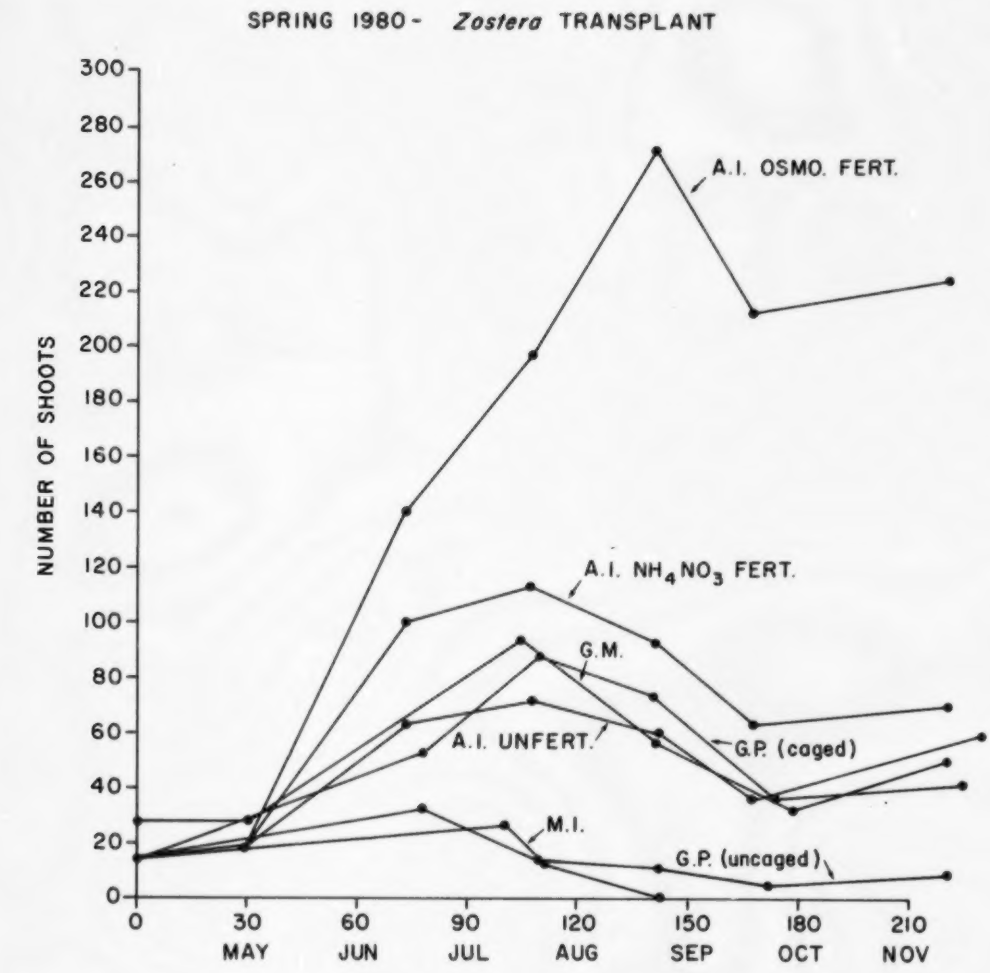

Fig. 6. Mean number of shoots vs. time for Spring, 1980, Zostera marina transplants.

previously caged Z. marina plugs had expanded an average of 14 percent in area but had increased nearly 75 percent in numbers of shoots per plug. The uncaged plugs on the other hand decreased an average of twelve percent in area and 23 percent in numbers of shoots during this same period.

From July to August both sets of Spring 1980, transplants at the Gloucester Point site underwent their typical summer dieback as water temperatures averaged nearly 30°C and light attenuation reached near maximum levels (Fig. 5). The mean areas of the plugs increased as the individual shoots spread apart, due in large part to the separation of the rhizome networks, while the mean number of shoots per plug decreased slightly. The average caged plug still had five times the number of shoots as compared to the uncaged transplants. By September 22, both sets of transplants had decreased nearly 50 percent in numbers of shoots from their August levels. However, water temperatures after this time dropped below 20°C and light attenuation decreased dramatically so that by November 13, 1980 new growth was evident in both the caged and uncaged treatments. At this time the average plug from which the mud snails had been excluded in the spring, had three times the number of shoots of its snail impacted counterpart.

In contrast to the three downriver stations the Mumfort Island site was the only location none of the vegetation survived the summer. These results are similar to that of the spring 1979, transplants placed here. As with the Gloucester Point site mud snail infestations became severe in April but continued for a slightly longer period until late June. There were no plugs protected from the snails by the cages at Mumfort Island. By July 10, 1980 the snails had left the vegetation and observations indicatd a loss of approximately one third of the plugs, a nearly six fold spreading in the mean areas of the plugs with an approximately two fold increase in the number of shoots per plug. Thus some growth had continued despite the apparently severe impact of the snails. After July 10 however, a precipitous decline ensued, such that by August 26, all the remaining vegetation had died.

Patterns of growth and decline of the fall Zostera marina transplants closely follow that of water temperaturs. At temperatures below 25°C survival of the plugs is excellent with growth occurring primarily when temperatures are betwen 10°C and 20°C (Fig. 7). These patterns of growth are very similar to those observed by Setchell (1929) in his early studies of Z. marina. Transplantation of Z. marina at most sites during the summer when water temperatures are above 25°C resulted in a significant decline of the vegetation at most sites. Transplantation during September and October when temperatures were 25°C or less resulted in little mortality until the following summer. Transplantation during the spring resulted in growth until temperatures again approached 25°C. A compounding factor to this observation of temperature stress is the fact that not all of the sites responded similarly to the high temperatures. Although there was no observable difference between the temperatures at each of the sites, the summertime declines occurred earlier and were more severe the further upriver the transplants were made. This suggests another factor or factors that may be acting synergistically with temperature controlling the survival of the plants.

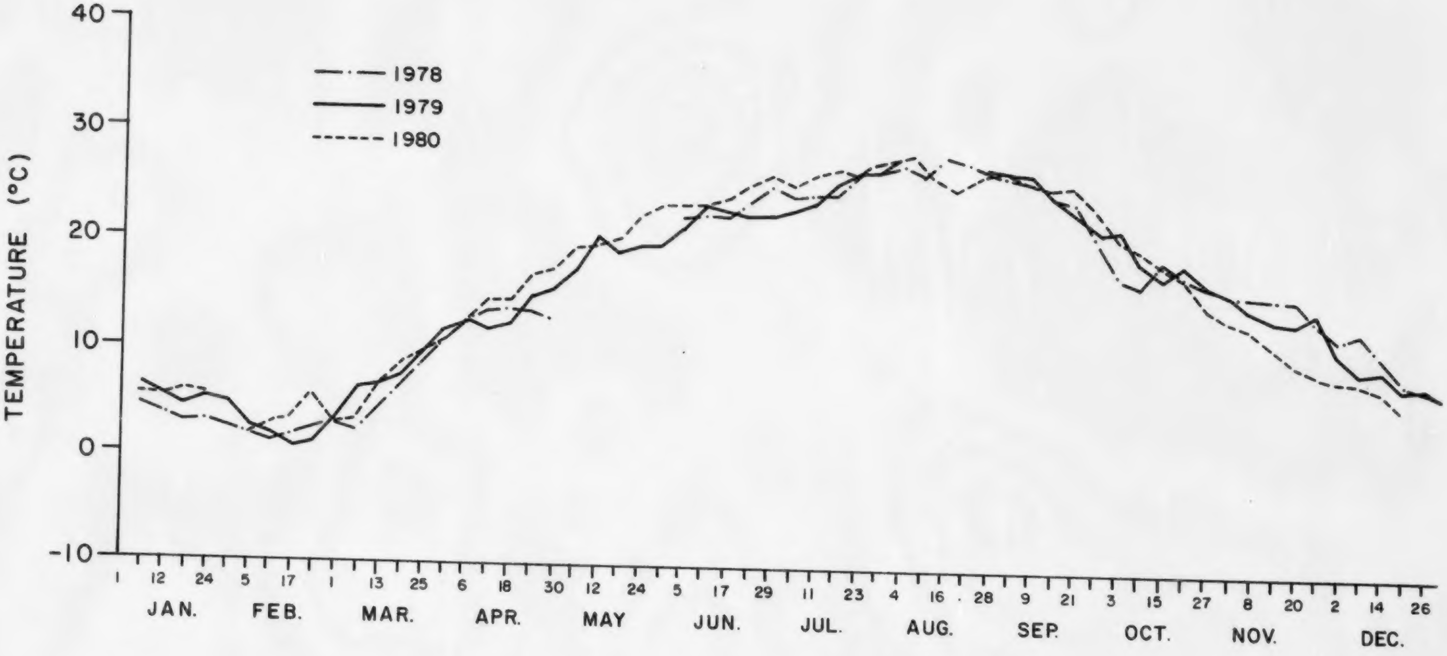

Fig. 7. Averaged daily York River water temperatures for 1978, 1979, 1980 at Gloucester Point, Va.

Salinity is a parameter that generally decreases with distance upriver in estuarine systems such as the Chesapeake Bay and its tributaries. However, salinities were generally quite comparable at each of the York River sites and only slightly less at the Parrott Island location. In addition, although low salinities can limit the survival of Zostera marina plants the periods of summertime decline observed here were generally characterized by increasing salinity at all sites.

Biological impacts from organisms such as Illyanassa obsoletus, the mud snail, were most severe in the upstream York River areas of Gloucester Point and Mumfort Island. Exclusion of the snails by the use of cages at the Gloucester Point location significantly increased the growth and survival of the plugs here. The snails are definitely a stress to the transplants, however, in most cases the decline of the vegetation occurred sometime after the snails had left the vegetation. In addition, the Parrot Island site which had the most severe and rapid loss of vegetaation of any of the areas had no significant infestation of snails. This suggests that although the mud snails can decrease the growth and survival of transplanted vegetation they are not solely responsible for the summertime losses at the upriver sites.

Daily mean attenuation coefficients taken from June to November 1980, at the Guinea Marsh, Allens Island, Gloucester Point and Mumfort Island transplant site are presented in Figures 8, 9, 10 and 11, respectively. Patterns of light attenuation illustrated by this data suggest significantly less attenuation during the summer months at the Guinea Marsh site as compared to the other areas. In addition, light attenuation remained relatively constant throughout the study period at Guinea Marsh compared with an increase in attenuation from June through September (at the other three sites) followed by a rapid decrease in attenuation during October and November. The Mumfort Island and Gloucester Point sites had nearly identical patterns of attenuation, suggesting no significant difference between the sites. However, the Allens Island area, although showing a similar pattern to these two sites, did not reach as high a peak in attenuation and showed considerably clearer waters in November. Considering the reduction in survival of the Zostera transplants as a function of distance upstream and the apparent increase in light attenuation along the same horizontal gradient it is suggested that Zostera transplants already stressed by high temperatures may be synergistically affected by decreased light quantity. Further we can hypothesize that severe reductions in available light during periods of high temperature stress may have significantly affected the viability of established beds in these curently denuded areas.

CONCLUSIONS

1. Comparisons of the two methods of transplanting Zostera marina in the Chesapeake Bay reveals cost per acre of $8,000 for the use of plugs versus $42,000 for woven mats. Costs per shoot are $0.07 and $0.38, respectively. The cost differential is largely that of the labor required to place the individual Zostera marina shoots into the biodegradable mesh. The use of plugs requires the transportation of

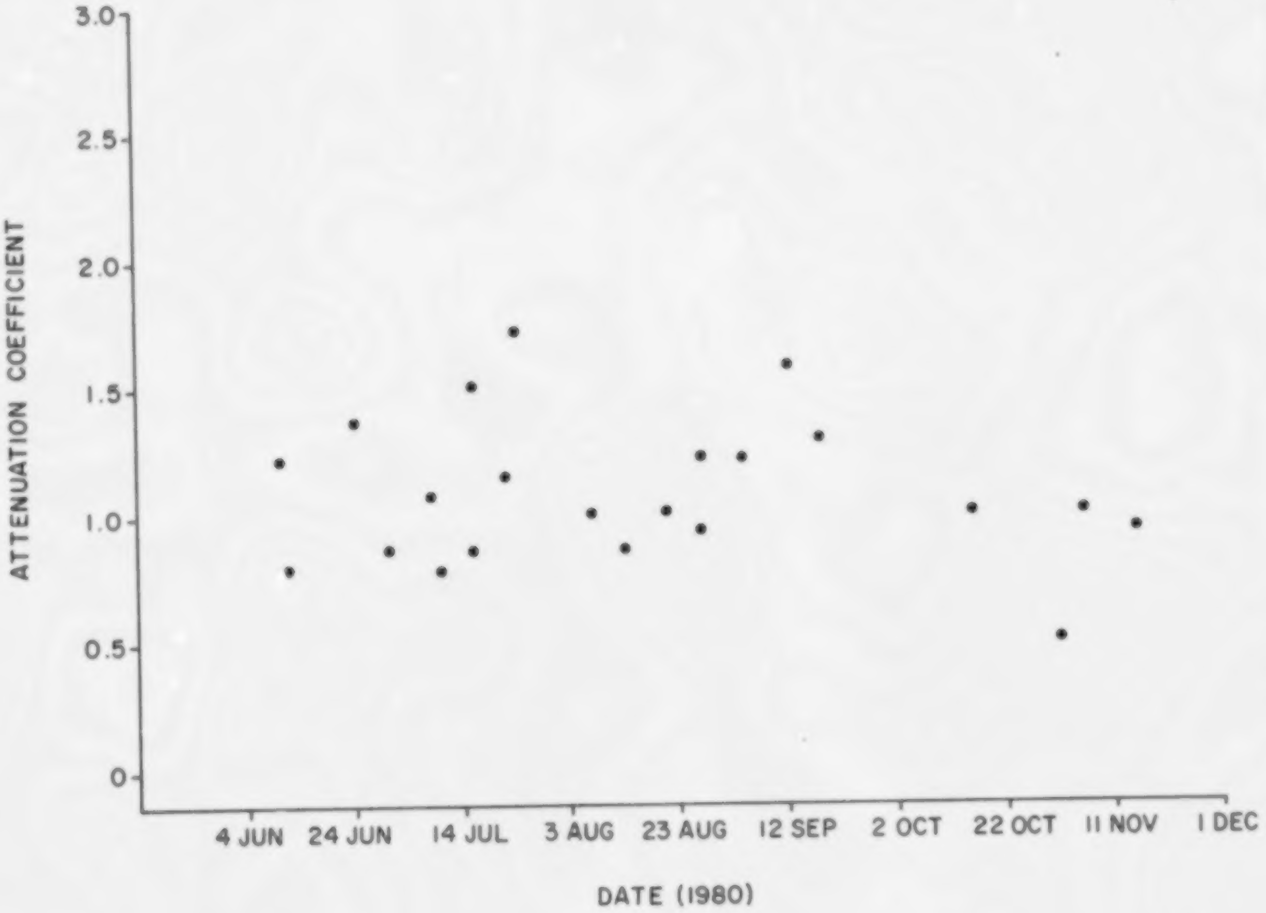

Fig. 8. Mean daily attenuation coefficients (Kd) for Guinea Marsh donor and transplant site.

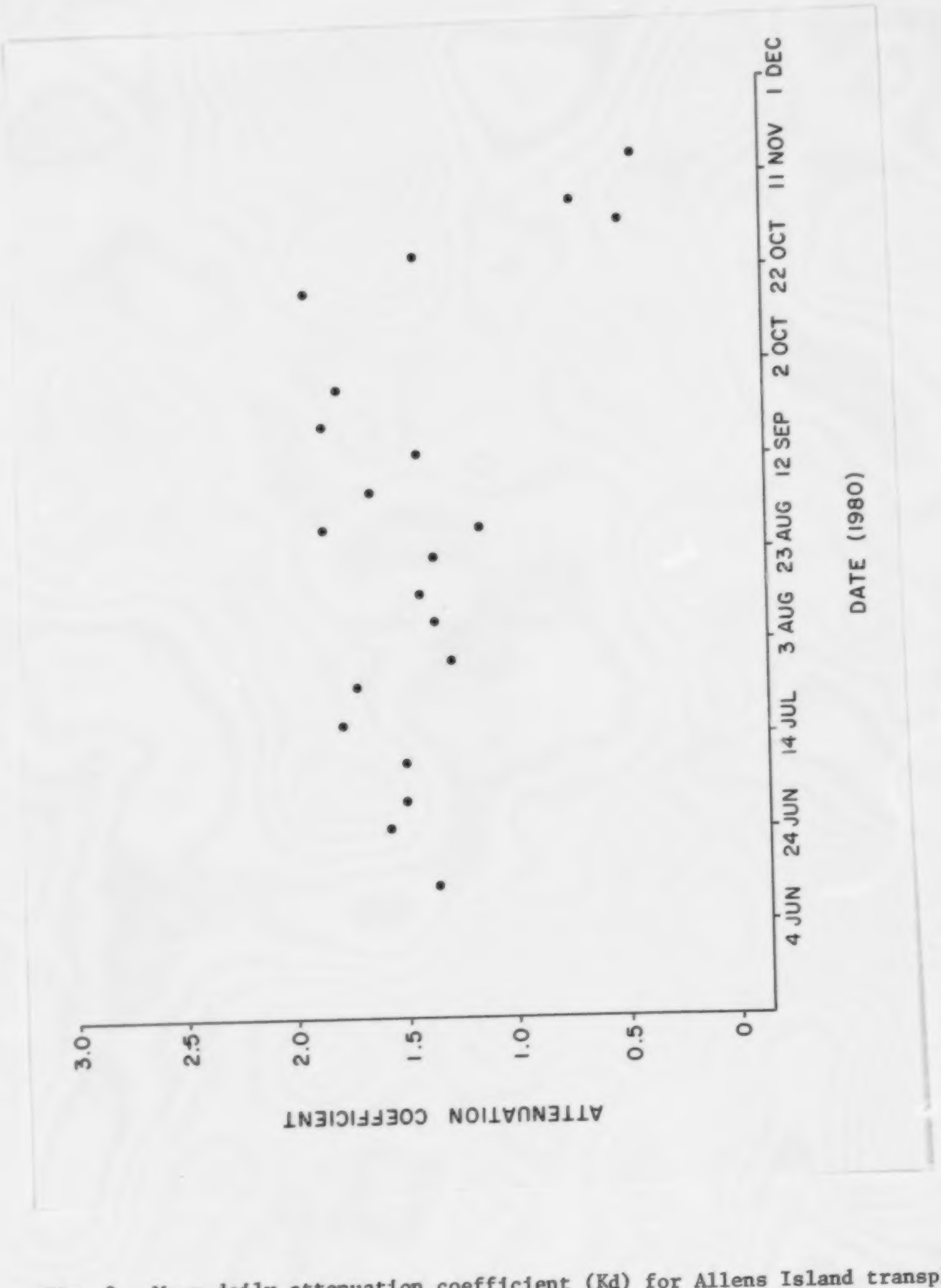

Fig. 9. Mean daily attenuation coefficient (Kd) for Allens Island transplant site.

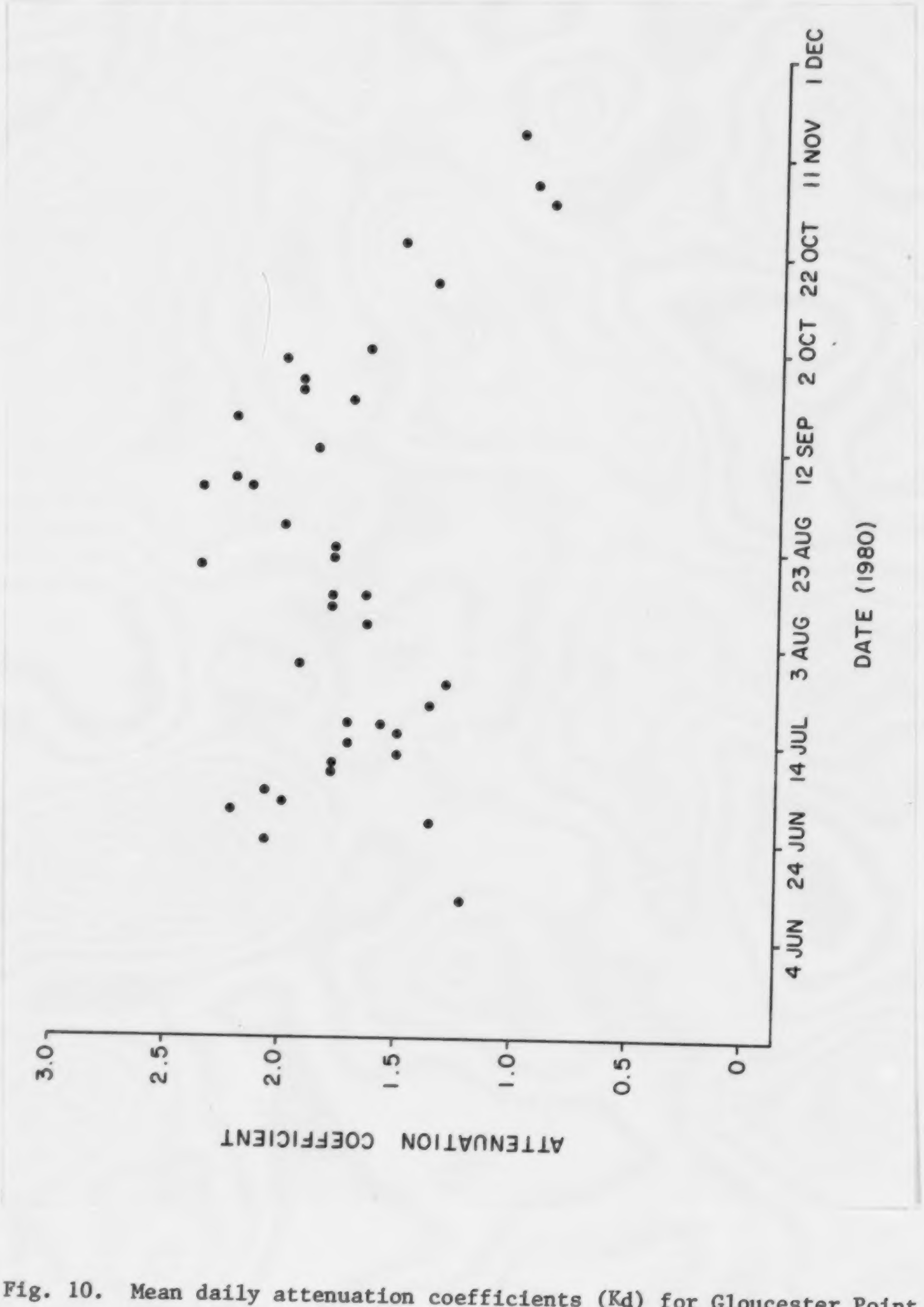

Fig. 10. Mean daily attenuation coefficients (Kd) for Gloucester Point transplant site.

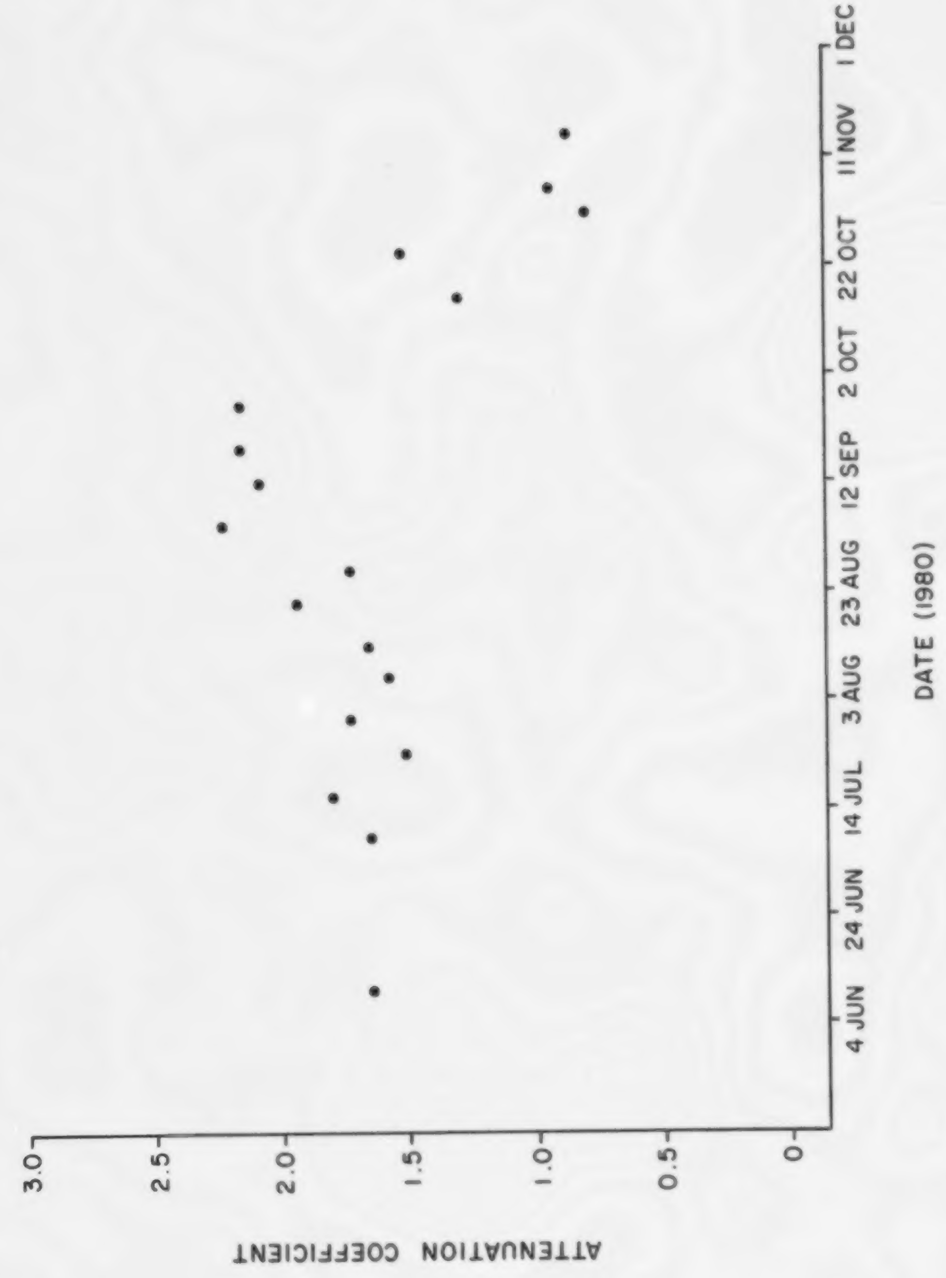

Fig. 11. Mean daily attenuation coefficients (Kd) for Mumfort Island transplant site.

large amounts of sediment but the resultant intact root structure provides an excellent anchor in the typically high energy transplant sites.

2. Transplantation of Zostera marina by the use of plugs of wild plants in the lower Chesapeake Bay is a viable management option for mitigation in regions that currently have existing vegetation. Transplantation is feasible in these areas during the summer, fall and early spring periods, but greatest survival has been demonstrated in the fall, followed by the spring, with least survival of those transplanted during the summer. Transplant of Zostera marina into regions currently denuded of vegetation can be attempted during the fall, although survival through the following summer may be minimal.

3. Location of a transplant site is critically important to the survival of the vegetation. In most cases areas to be transplanted must have previously supported Zostera marina beds and have depths between 0.5 and 1.0 m at MLW. Survival of transplanted areas is inversely related to the distance upriver from areas of existing vegetation, with the poorest chances for success in those areas where Z. marina historically has experienced its most upriver limits.

4. The use of ammonium nitrate fertilizer (37-0-0) implanted at 10 to 15 cm depths in the sediment under the transplanted plugs had no significant effect on the growth of plugs transplanted during the summer and fall periods. It did increase the growth of transplants in one area where established vegetation was present during the spring of 1980. Its use is not recommended. Osmocote fetilizers (14-14-14) used in a similar manner at the same location and time resulted in significantly greater growth of the Zostera marina. Its use is recommended.

5. Monitoring the growth and survival of the Zostera marina transplants during this study has revealed that dieback begins in the farthest upstream sites when temperatures reach 20°C by approximately June 1. Declines begin later in the downriver areas as temperature reaches 25°C. The stressful period ends as temperatures drop to between 20°C and 25°C during September. The longer the period of time that the Zostera marina can be transplanted before these high temperatures are reached, the greater the success rate.

6. The greater average light extinction observed in the upriver areas along with poorest survival rates at these sites during the summer suggest that reductions in available light may be acting synergistically with high temperatures to limit the growth of the transplanted vegetation and to control natural regrowth. Abnormally high reductions in available light, combined with high summertime water temperatures may have been responsible for the recent rapid loss of natural vegetation from many of these now denuded areas.

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CHAPTER 5

REGROWTH OF SUBMERGED VEGETATION INTO A RECENTLY DENUDED BOAT TRACK

by

Kenneth A. Moore
and
Robert J. Orth

ABSTRACT

Patterns of regrowth of the submerged macrophytes, *Zostera marina* and *Ruppia maritima* into a recently denuded boat track were observed during a seven month period. Revegetation occurred primarily by lateral growth from the unimpacted vegetation at the sides of the cut with *R. maritima* being the more rapid colonizer. Growth from *Z. marina* seedlings observed during the fall months while *Z. marina* shoots not completely removed from the sediment by the boat propellor served as other foci for regrowth throughout the study period. Analysis of the sediments both inside and outside of the cut revealed little difference in the sediment grain size or pore water nutrient concentrations, indicating that the sediment characteristics were probably not a factor limiting regrowth into the denuded area. It is suggested that recolonization of a one meter wide boat track by *R. maritima* will take at least two seasons while recolonization by *Z. marina* will take three or more years.

INTRODUCTION

Beds of submerged vegetation are directly disturbed in many ways by man's activities (Zieman, 1976; Churchill et al., 1978). Dredging and filling associated with a need for deep water access to upland development projects may cut directly through established grass beds. In many cases, especially in the Chesapeake Bay region, proper planning in conjunction with both federal and state regulatory procedures can reduce or eliminate these impacts. Illegal dredging or other inadvertant disturbances are not as readily controlled.

Such inadvertant disturbances as boat tracks are commonly observed throughout the beds of submerged vegetation found in the lower Chesapeake Bay (Fig. 1). Although isolated events, in many instances they may significantly alter the bottom in areas where boating traffic is highest, primarily during April to October. These denuded tracks are primarily caused by propellers digging into the bottom while vessels traverse the beds during low tidal periods. The denuded areas can vary greatly in size, from a few decimeters to over a meter in width, and from a few meters to many hundred meters in length. The size is dependent upon a number of factors such as water depth, vessel size and operator concern or awareness.

Zieman (1976) indicates that in southern Florida physical damage from motor boats on turtle grass beds (*Thalassia testudinum*) persists from 2 to 5 years and that new vegetative growth by *Thalassia* into the cuts is very limited. He indicates, however, that Jones (1968) and Phillips (1960) report rapid recolonization by *Halodule beaudettei* in areas where it co-occurs with *Thalassia*. There is little reported evidence on patterns and mechanisms of regrowth onto similar denuded tracks found in the eelgrass (*Zostera marina*) and widgeon grass (*Ruppia maritima*) dominated beds which are found throughout the lower half of the Chesapeake Bay.

The object of this project was to observe the natural regrowth of vegetation into a boat track in a bed of submerged vegetation in the lower Chesapeake Bay. A large boat track was observed in May 1980, to have been formed across a SAV bed in the Brown's Bay region of the Mobjack Bay since a previous month's visit to the site in April 1980. The bed is approximately 500 m wide at this location and is part of a fringe of grasses found in the shallow (<2 m) littoral zone of the Mobjack Bay (Orth et al., 1979). *Ruppia maritima* dominates the shallow inshore zone (<-40 cm, MLW at this area) with *Zostera marina* dominating the deeper offshore zones (>-80 cm, MLW). Intermediate depths (-40 to -80 cm, MLW) are characterized by a mixture of the two species (Orth et al., 1979).

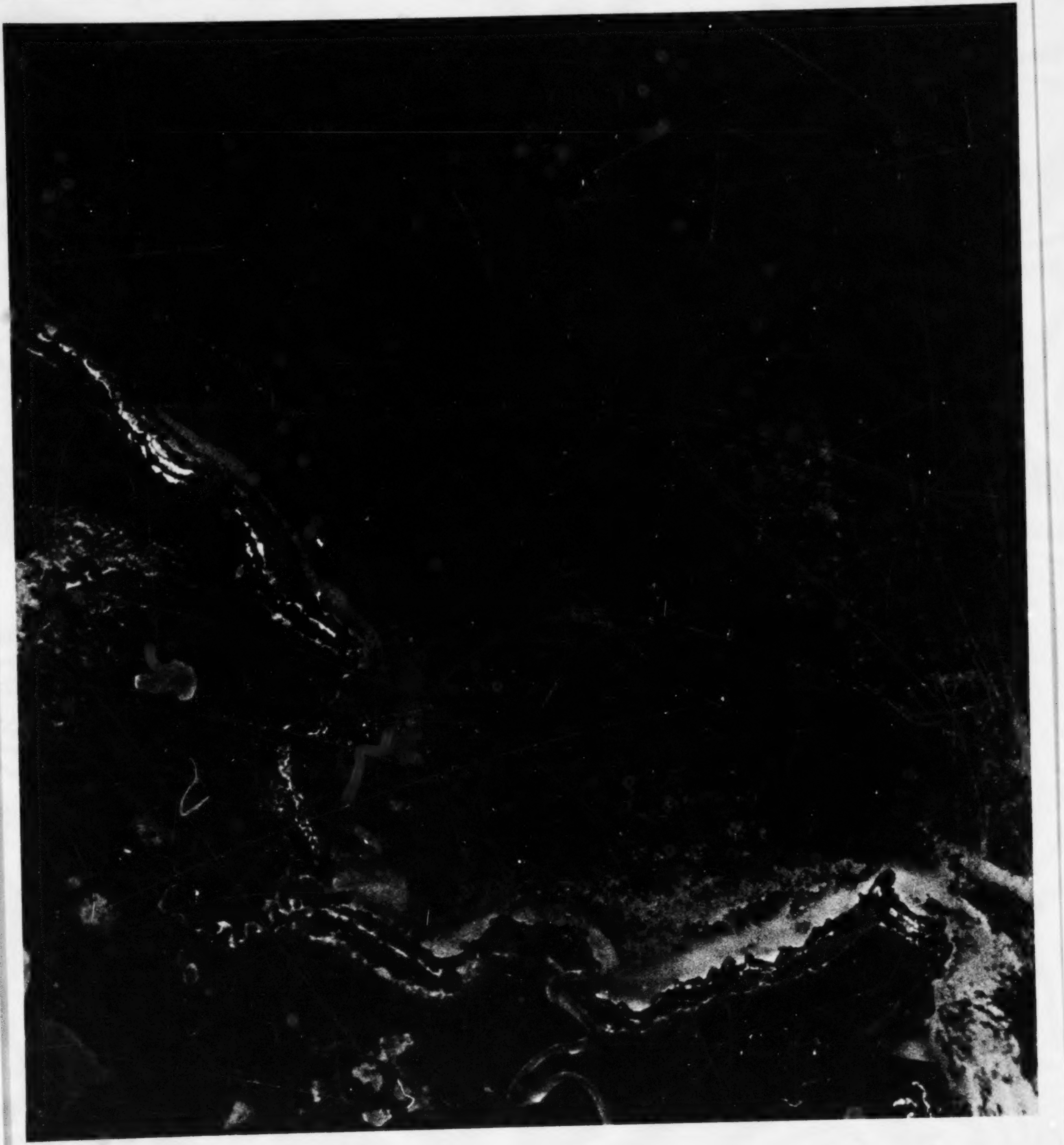

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Fig. 1. Brown's Bay, Virginia, SAV bed showing evidence of boat tracks.

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The boat track, when first observed, averaged approximately 1 m in width and extended in nearly a straight line for over 200 m throughout the mixed zone of the bed. It was oriented in nearly a 45° angle with the shoreline that is composed of an extensive saltmarsh dominated by Spartina alterniflora. Considering the size of the denuded area and the depth of water (-0.5 to -0.75 m, MLW) the cut was probably formed by a commercial crab potter or haul seiner with a moderately sized (30 ft.), inboard powered, dead-rise type vessel. Early in the season each year, crab potters place their pots largely within the grass beds of the Mobjack Bay. As a result, many of these beds are crisscrossed with unvegetated paths caused by heavy boating activity (Orth, 1976).

MATERIALS AND METHODS

Two approaches were used to monitor the regrowth of vegetation into the denuded boat track. In the first, a one meter square reference plot was staked out in the denuded area where the cut was found to be exactly one meter wide. Monthly observational data was obtained by a diver including percent of bottom revegtated, length and pattern of regrowth into the plot, recolonization by seedlings, etc. In addition, replicate sediment cores were obtained for analysis of particle grain size and interstitial nutrients both within the reference plot and one meter on either side of the cut in the unimpacted, vegetated area. The sediment cores were obained on June 11, 1980, and were repeated for particle size analysis only on November 23, 1980, at the end of the study period reported here. In addition to the data obtained on the reference plot, general observations were made by a diver at approximately monthly intervals over the entire length of the boat track. Such data included patterns of revegetation, changes in bottom by scouring or bioturbation, changes in orientation of cut, etc. as well as other qualitative observations. Temperature, salinity and PAR light readings were also obtained.

The sediment cores were obtained by use of 5 cm O.D. plexiglass core tubes 50 cm in length and graduated in cm increments. The tubes were forced into the bottom to a depth of approximately 30 cm, plugged with a rubber stopper and pulled from the bottom with the core tube containing the sediment, the vegetation (if present) and the overlying water. The tubes were capped at the top and bottom while still submerged and removed to a covered container filled with ambient temperature seawater. Immediately after all samples were taken the core tubes were returned to the lab for extraction.

Upon return to the lab each core tube was uncapped at the top and 100 ml of the overlying water extracted using a large hypodermic syringe with an attached 0.45 μ fiber filter in a filter holder. The filtrate was placed in a 50 ml plastic, conical centrifuge tube with a screw cap and immediately frozen for later analysis. The sediment plug, including plant shoots, roots and rhizomes, was extruded from the core tube onto a graduated holder and sectioned into 0-2, 2-5, 5-10, 10-15 cm depth segments. Each segment of plug sediment was placed in a Gelman filter centrifuge tube holder and centrifuged for 10 minutes through a 0.45 μ glass fiber filter. The filtrate was transferred to a 50 ml capped centrifuge tube and immediately frozen. In addition, the sediments of each depth interval of each core were placed in Whirl-paks and immediately frozen for later grain size analysis through standard pipette and dry sieving techniques (Folk, 1961). Pore water from each segment and the sample from the overlying water were analyzed for NH_4^+, NO_3^-, NO_2^- and PO_4^{-3} using automated analysis techniques (EPA, 1974) with a technitron auto-analyzer. Modifications to these techniques were made after Wetzel et al., 1979, including concentration of nitrate/nitrite reagents, a two reagent chemistry for phosphate determination and a two reagent chemistry for phosphate determination and a two reagent chemistry for ammonia (Solorzano, 1969; Koroleft, 1970; Gravitz and Gleye, 1975; Liddicoat, Tibbits and Butler, 1975).

RESULTS AND DISCUSSION

Observations made during the May 23, 1980 visit to the Brown's Bay area revealed that the entire length of the denuded boat track, including the test plot (Fig. 2), was characterized by the presence of only a few scattered Zostera marina seedlings and small patches of Z. marina shoots growing from remaining sections of rhizomes. Apparently the boat propeller had effectively uprooted nearly all the Z. marina. Similarly, there was virtually no Ruppia maritima within the denuded zone. There were however, numerous examples of new growth of R. maritima spreading from the adjacent vegetation portions of the bed. The growth consisted of straight rhizome runners up to 15 cm in length with new shoots at several cm intervals. In contrast, there was very little evidence that Z. marina was spreading from the adjacent vegetated areas.

Triplicate 0.033 m^2 cores were taken from an adjacent unimpacted section of the Brown's Bay submerged grass bed on May 19, 1980. Complete data from this sampling are presented in Chapter 1 of this report. The data indicate means of 100 g/m^2, 76 g/m^2 and 136 g/m^2 for standing stock of Zostera marina vegetative shoots, reporductive shoots and roots and rhizomes, respectively. Total Ruppia maritima standing stock was found to average 24 g/m^2. Assuming that these data are representative of the vegetation that would have been growing within the denunded area at this time and that the area itself measured 200 m x 1 m, then a dry weight standing stock of approximately 20 kg of Z. marina vegetative shoots, 15 kg of Z. marina reproductive shoots, 27 kg of Z. marina roots and rhizomes and 5 kg of R. maritima shoots, roots and rhizomes were essentially missing in May 1980 as a result of an apparent single pass by a motor boat in April 1980. These equate for Z. marina to nearly 340,000 vegetative shoots and 85,000 reproductive shoots. Although data on densities of R. maritima shoots are not available for this Brown's Bay area, calculations were made using a shoot density to total biomass ratio determined for a similar mixed species zone at a Vaucluse Shores sampling station (Chapter 1). They indicate that approximately 1.2 million R. maritima shoots could have been growing during May 1980, in this now denuded 1 x 200 m boat track.

The bottom within the track during May was for the most part quite flat in cross sectional view. In most areas of the cut, including the 1 m^2

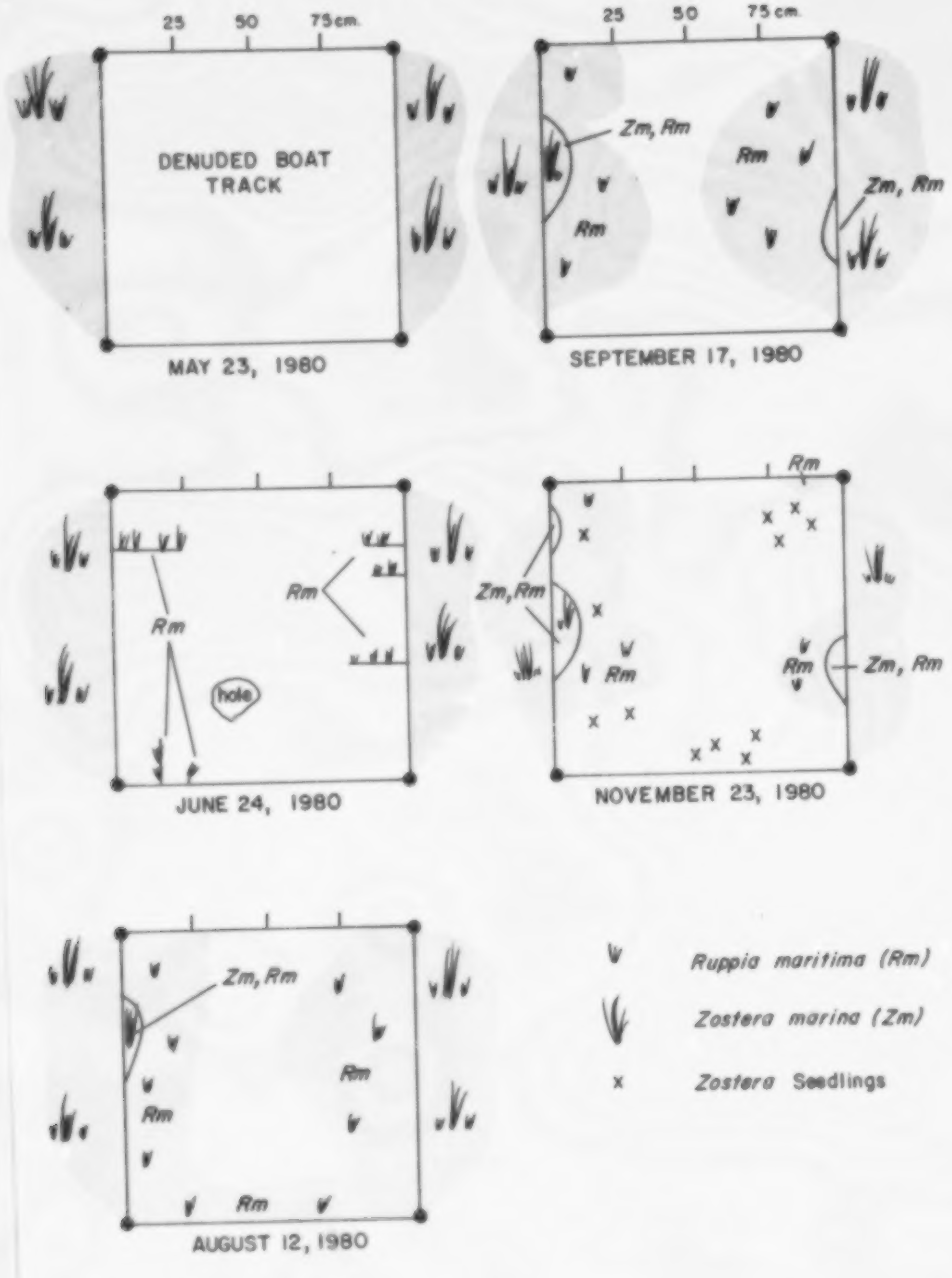
25
50
75 cm.
DENUDED BOAT TRACK
MAY 23, 1980
25
50
75 cm.
Zm, Rm
Rm
Rm
Zm, Rm
SEPTEMBER 17, 1980
Rm
Rm
hole
JUNE 24, 1980
Rm
Zm, Rm
Rm
Rm
Zm, Rm
NOVEMBER 23, 1980
Zm, Rm
Rm
Rm
Rm
AUGUST 12, 1980
Ruppia maritima (Rm)
Zostera marina (Zm)
Zostera Seedlings

Fig. 2. Regrowth of submerged vegetation into the test plot.

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reference area, the bottom was of similar depth to the adjacent, unimpacted bottom although at several locations it did appear that several cm of sand had been removed or eroded from the cut. After intensive storm events, however, similarly formed boat tracks have been observed to lose considerable amounts of material through scour by wave and current action (personal observation). This condition would be more similar to what Zieman (1976) observed in his study of Thalassia testudinum beds.

In numerous areas along the edges of the denuded cut and adjacent to the existing vegetation, 10 to 20 cm diameter holes had been excavated to depths of 15 to 20 cm. These holes which extended under the Zostera marina and Ruppia maritima exposing both roots and rhizomes, were apparetly dug by both blue crabs and toadfish. Orth (1975) reported similar features in artificially clipped plots within comparably vegetated beds in this region.

Particle size distribution, in percent, for the sediments within the 0-2, 2-5, 5-10 and 10-15 depth intervals ae presented in Table 1 and statistical parameters of grain size in Table 2 for replicate cores taken both inside and outside of the cut on June 11, 1980. Graphic mean (M_2) and median (Md) measures of average size indicte that sediments are quite similar with respect to depth. Although the use of only replicate sampling did not allow for a good measure of variance, ANOVA revealed no significant effect of depth and no difference between inside and outside of the boat track. Core #1 taken outside the boat track did show a larger percent of material in the 0 phi size class of the 0-2 cm core section. There is little to suggest from these data that now there was a significant effect of the boat propeller on the sediment. The inclusive standard deviation or sorting coefficient (σ_I) indicates that the sediments are moderately sorted at all depths both inside and outside of the track. The inclusive graphic skewness measure (Sk_I) reveals the sediments to be fine-skewed to strongly fine-skewed with no effect of depth or location. These results are in contrast to data of Zieman (1976) who suggests a decrease in fine material (4 phi) in a single boat track as compared to the unaffected Thallasia testudinum bed. It would appear that the considerable mechanical disturbance of the boat propeller which was capable of removing nearly 100 percent of the vegetation had little observable effect of the grain size distribution of the sediments by June 1980.

Extractable interstitial nutrient concentrations for the sediment cores are presented in Table 3. Data for ammonium indicate higher levels at depths below 10 cm within the boat track when compared to outside. This suggests that ammonium produced by mineralization of organic nitrogen plus other processes may be accumulating due to lack of uptake by plant roots. Reduced levels of ammonium in the surface layers both within the outside of the denuded track relative to the submerged layers suggest oxidation of ammonia to nitrite and nitrate may be occurring at these shallow depths. Diffusion of ammonium into the water column would also contribute to reduced concentrations nearer the surface. Lower levels of ammonium in the 0-2 and depth segments were found inside the boat track as compared to outside. Greater perturbation of surface of the sediments within the denuded cut by waves or organisms such as blue crabs, etc. might lead to greater losses of the ammonium when compared to the more protected vegetated areas. The

TABLE 1. PARTICLE SIZE DISTRIBUTION, (%) FOR SEDIMENT CORES TAKEN INSIDE AND OUTSIDE OF BOAT TRACK, 6-11-80.

Core	Depth (cm)	(mm) φ	1.000 0	.500 1	.250 2	.125 3	.063 4	<.063 5
Out-1	0-2		3.65	1.55	17.26	56.09	7.52	15.12
"	2-5		1.50	1.90	45.60	36.45	4.19	10.40
"	5-10		0.57	1.98	44.44	43.30	2.70	9.20
"	10-15		0.48	2.03	41.43	40.85	3.40	11.20
Out-2	0-2		0.11	1.22	58.54	30.50	1.69	7.87
"	2-5		0.18	2.78	49.49	36.30	3.13	7.30
"	5-10		2.24	2.26	41.92	39.80	4.90	9.09
"	10-15		0.16	1.63	67.83	22.46	1.50	6.36
In-1	0-2		0.06	0.62	45.50	44.06	2.40	6.85
"	2-5		0.21	1.77	51.34	37.40	2.30	6.40
"	5-10		0.47	2.50	43.32	43.60	3.20	7.20
"	10-15		0.64	2.58	48.03	38.35	2.03	7.77
In-2	0-2		2.40	1.30	31.80	50.87	4.80	8.70
"	2-5		1.09	1.04	40.84	45.45	4.30	7.40
"	5-10		1.23	1.58	36.80	48.20	4.74	7.86
"	10-15		1.42	1.91	49.26	37.10	2.85	7.40

TABLE 2. STATISTICAL PARAMETERS OF GRAIN SIZE FOR SEDIMENT CORES TAKEN INSIDE AND OUTSIDE OF BOAT TRACK, 6-11-80.

Core	Depth (cm)	Mean (M_z)	Median (Md)	Sorting (σI)	Skewness (SK_1)
Out-1	0-2	2.6	2.5	1.00	+0.12
"	2-5	2.1	2.0	0.92	+0.35
"	5-10	2.1	2.1	0.76	+0.23
"	10-15	2.1	2.1	0.76	+0.23
Out-2	0-2	2.3	2.3	0.77	+0.10
"	2-5	2.2	2.2	0.76	+0.31
"	5-10	2.2	2.1	0.74	+0.16
"	10-15	2.1	2.0	0.77	+0.32
In-1	0-2	2.0	1.9	0.72	+0.46
"	2-5	2.1	2.0	0.80	+0.27
"	5-10	2.1	2.1	0.85	+0.18
"	10-15	1.9	1.8	0.77	+0.30
In-2	0-2	2.2	2.1	0.71	+0.39
"	2-5	2.1	2.0	0.74	+0.30
"	5-10	2.0	2.1	0.77	+0.28
"	10-15	2.1	2.0	0.80	+0.27

TABLE 3. SEDIMENT PORE WATER NUTRIENT CONCENTRATIONS (μM) IN CORES TAKEN INSIDE AND OUTSIDE OF BOAT TRACK, 6-11-80.

Core	Depth (cm)	NH_3^+	NO_3^-	NO_2^-	PO_4^{-3}
Out 1	water	1.16	0.04	0.16	0.58
"	0-2	41.5	2.38	1.56	4.23
"	2-5	62.8	<0.01	1.48	8.99
"	5-10	59.4	0.25	1.40	0.39
"	10-15	64.3	0.04	1.65	9.31
Out 2	water	0.70	0.07	0.20	0.61
"	0-2	25.6	7.07	4.93	17.3
"	2-5	44.1	5.40	2.48	18.5
"	5-10	51.2	3.29	1.56	12.9
"	10-15	38.2	2.38	1.48	11.0
In 1	water	0.44	0.08	0.16	0.36
"	0-2	8.88	5.48	5.10	9.31
"	2-5	54.1	0.82	1.48	6.70
"	5-10	151	1.64	1.56	15.6
"	10-15	135	3.95	1.82	16.4
In 2	water	0.34	0.08	0.21	0.36
"	0-2	15.7	5.49	2.74	4.94
"	2-5	17.9	<0.01	1.23	4.66
"	5-10	49.8	<0.01	1.23	2.34
"	10-15	133	<0.01	1.48	6.62

characteristic tan color of the oxidized horizon was observed to depths of 3 cm in the boat track but only 1 cm in the vegetated area.

Data for nitrate and nitrite indicate highest concentrations in the 0-2 cm layers. This seems reasonable assuming these levels are largely products of the upward diffusion and oxidation of ammonium as described by Gambrull and Patrick (1978) for flooded soils. Lower concentrations for these inorganic nitrogen species are observed below 2 cm depths. This may be attributed to the lack of nitrification as well as to their loss under these reduced conditions through the denitrification pathway as molecular nitrogen or nitrous oxide. In contrast to the reduction in ammonium levels, there appears no evidence that concentrations of nitrate are lower in the vegetated area below 5 cm depths when compared to the unvegetated boat track.

Inorganic phosphorus concentrations in the sediments were relatively constant with depth and we were unable to observe a gradient between the deeper anaerobic sediments and the oxidized surface horizons. This is not unexpected since, as described by DeLaune, Patrick and Brannon (1976), phosphate is not directly involved in oxidation-reduction reactions in flooded systems, but its solubility is related to the state of the ferrous/ferric iron system as well as other factors.

We could find little difference between concentrations of extractable phosphate in the sediments of the vegetated cores taken outside the boat track and the unvegetated cores taken within. Potentially, phosphate levels in the interstitial water could be less in the vegetated cores due to plant uptake of precipitation as insoluble ferric phosphate around the oxidized rhizosphere. Lack of a significant difference between the two areas suggests that during this sampling period the sediments were supplying adequate phosphate to overcome any plant uptake or precipitation.

On June 24, 1980, observations made along the boat track revealed that Ruppia maritima had rapidly extended from the side of the cut and in several areas had expanded up to one third of the distance across the track. In contrast to the straight rhizomes observed in May, the R. maritima had branched out to form small patches of vegetation 15 to 20 cm in diameter. Zostera marina was scattered but very sparse in abundance throughout the boat track. The Z. marina consisted mainly of isolated rhizome segments several up to 20 cm long but most less than 10 cm in length with 3 to 4 vegetative shoots. They appeared to be formed primarily from the growth of sections of rhizome not completely removed by the boat's propeller as well as from seedling growth.

The bottom topography of the denuded zone was much more irregular than that observed in May. There were many more depressions, some apparently recently dug by blue crabs, with nearly vertical sides and depths to 10 cm. Others appeared to be older and had filled in to varying degrees. Each had a characteristic mound of sand piled adjacent to the hole, a result of the digging activity (Orth, 1975; Dunnington, 1956). Adjacent vegetated areas had similar holes scattered throughout but in greatly reduced density. It appeared that the Zostera marina and Ruppia maritima rhizome mat was an effective inhibitor of the digging activity (Orth, 1977).

The sediment surface within the boat track was also littered with mats of *Zostera marina* shoots. Most were the typical sloughed off, brownish, vegetative leaves. However, some consisted of whole green plants, apparently recently uprooted, complete with rhizomes. Since the flowering period for *Z. marina* had just ended, a few decaying reproductive shoots were located, although no seeds were found in the spathes. Much of this detrital material had accumulated in the numerous depressions in the bottom and in many instances was being covered by sand from the slumping of the sides of these holes.

We observed a significant expansion of *Ruppia maritima* into our test plot during this period from the adjacent vegetated zones (Fig. 2). The recolonization was characterized by new growth at three locations extending 5 to 25 cm from the sides of the cut as straight rhizomes with a few lateral branches. No significant revegetation by *Zostera marina* was evident. A crab hole approximately 10 cm in diameter by 10 cm deep had been dug in the center of the plot but otherwise the plot had been undisturbed.

On August 12, 1980, the boat track was characterized by large amounts of detrital *Zostera marina* vegetative shoots and *Ruppia maritima* reproductive shoots covering the bottom. This detrital material was found throughout the vegetated portion of the bed but was readily accumulated in the narrow, open boat track to thicknesses of 5 to 10 cm. The *Z. marina* within the bed was experiencing its typical, midsummer die-back and the leaves were heavily encrusted with thick deposits of epiphytic diatoms as well as algae, bacteria etc. as described by Sieburth and Thomas (1973) and Jacobs and Noten (1980). These heavily encrusted leaves are readily broken off. The *R. maritima*, although not as heavily encrusted as the *Z. marina*, was characterized by numerous long (1 m) reproductive shoots, many of which had been shed and were littering the bottom in much the same manner as the *Z. marina* reproductive shoots had been found the previous month.

The bottom within the boat track was much more regular in cross sectional view than that found during June, with fewer crab holes and other depressions. Revegetation by the lateral spreading of *Ruppia maritima* from adjacent vegetated areas onto the denuded boat track was continuing. In several areas, patches of *R. maritima* spreading from adjacent vegetated areas onto the denuded boat track was continuing. In several areas, patches of *R. maritima* spreading from both sides of the cut had nearly joined together, although in most sections *R. maritima* had revegetated 30 to 50 cm from the sides of the boat track. In contrast to the adjacent, undisturbed portions of the bed no reproductive shoots were observed among this new growth. Revegetation by *Zostera marina* was again much less pronounced than that of *R. maritima*. There appeared to be fewer patches of *Z. marina* within the denuded track during this July period than there was in June and regrowth was limited to a few areas where encroachment was only to 5-10 cm in width.

The one meter square staked area demonstrated the continued re-growth of *Ruppia maritima* across the boat track (Fig. 2). Nearly continuous bands of regrowth extending 50 cm from one side of the cut and 30 cm from the other were observed. As with other revegetated areas within the boat track no reproductive shoots were found. In contrast to the *Ruppia maritima*, *Zostera* *marina* again showed little evidence of extensive regrowth. In only one area did the *Z. marina* spread from the adjacent vegetated zone, and then for only a distance of 5 cm. The crab hole observed in this reference area in June had filled in and was not evident in August.

On September 17, 1980 observations made along the boat track revealed an apparently reduced growth rate by *Ruppia maritima* during the August-September period as compared to the July-August and June-July periods. In most sections of the denuded zone *R. maritima* was covering one-third to one-half of the originally impacted bottom. This is quite similar to observations made during the previous month. In several sections however, *R. maritima* patches from both sides of the cut had joined together to completely cover the bottom. In August these areas had not quite grown together. Regrowth of *Z. marina*, in comparison, was still characterized by only small isolated clumps of vegetation, either as monospecific stands or mixed with the more rapidly spreading *R. maritima* which had extended from the sides of the cut. Little significant spreading by the *Z. marina* was evident. Similar to observations made during August, abundant detrital *Z. marina* and *R. maritima* shoots were found throughout the bottom.

The staked one meter square reference area showed reduced coverage by *Ruppia maritima* when compared to the August observations (Fig. 2), but moderate expansion by *Zostera marina* was observed. This compares with an annual secondary period of growth observed for *Z. marina* in this region (Section 1). Along the west side of the cut a small area of *Z. marina* had extended an additional 5 cm from the edge of the vegetated, unimpacted zone. Along the east side several shoots of *Z. marina* were observed for the first time but only 2 cm from the side of the cut.

Final observations on the regrowth of the submerged vegetation into the boat track that is presented in this report were taken on November 24, 1980, six months after the initial sampling period and approximately seven months after the cut was made. At this time the boat track was still well defined and largely unvegetated. The bottom showed little evidence of active bioturbation by large organisms in contrast to the previous summer months. Little scouring of the boat track was evident with depths in the cut nearly comparable to the adjacent unimpacted areas. Wave-formed ripples approximately 2 cm high and at 10 cm intervals were evident throughout the unvegetated bottom.

Revegetation of the boat track was still quite limited. *Ruppia maritima* was observed to have spread completely across the cut at only three points throughout its 200 m length and appeared less dense than during September. In most areas the *R. maritima* was found to extend only 10 to 40 cm from the sides of the cut. There were however small isolated patches of *R. maritima*, consisting of 10 to 15 shoots, scattered throughout the unvegetated zone. These were probably remnants of *R. maritima* which had spread from the sides of the boat track as opposed to new growh surrounding *R. maritima* seedlings.

There were however numerous *Zostera marina* seedlings found for the first time throughout the boat track. For the most part they ranged from 5 to 8 cm in height and contained 2 to 3 leaves per plant. *Z. marina* seeds in this region are found to germinate beginning in the fall and continuing throughout the winter into the spring months (Chapter 3).

The spreading of Zostera marina from the sides of the cut did not appear significantly greater than in September. In one area of the boat track spreading Z. marina had reached 40 cm from the edge of the cut, but otherwise it appeared the Z. marina had intruded on the average only 5 to 10 cm.

The one meter square test plot paralleled the observations made for the entire boat track. The Ruppia maritima was reduced in coverage over that observed in September while the Zostera marina had not significantly expanded its coverage. Twelve Z. marina seedlings were found within the plot. This compares with a mean of 66 per m^2 found in the interior portion of a nearby Z. marina bed in February 1980. Z. marina seedlings of course are quite variable in their distribution, however as the winter continues we would expect more and more seedlings to be found.

Visual analysis of replicate sediment cores taken in November 1980, revealed both cores within the boat track were characterized by light tan sand to depths of 2 to 3 cm below the surface. Below this layer the sediment appeared of similar consistency to that above but was characterized by a grey color indicative of anaerobic conditions. Each core taken within the cut also had a 2 cm horizon, located at a depth of 10 cm, which contained decaying Zostera marina and Ruppia maritima roots and rhizomes as well as polychaete tubes and other organic matter. This loose material appeared to have been buried at this depth and no other roots or rhizomes were observed above or below in these cores. At approximately 20 cm of depth a characteristic distinct layer of sandy-clay was found. In one of the two cores a sample of this sediment found between 18 to 23 cm was analyzed for grain size.

Visual analysis of the two cores taken in the adjacent vegetated area revealed that a layer of light tan colored sand extended only to a depth of 1 cm. Below this, grey sand was found to approximately 20 cm depths where the increase in clay was evident. In contrast to the cores taken in the boat track, no distinct horizon or organic matter was found, however viable Zostera marina and Ruppia maritima roots and rhizomes were observed to 10 cm depths throughout the cores.

Particle size distribution in percent, for the 0-2, 2-5, 5-10 and 10-15 cm depth intervals of the sediment cores are presented in Table 4. Statistical parameters of grain size are found in Table 5. The 18-23 cm depth segment from core #2 taken inside the boat track reveals the characteristic sandy-clay layer found throughout this region. The fines (<5 phi) predominate in this layer, thus increasing the median and mean phi sizes significantly compared to the overlying sediments. The skewness measure for this layer of sediment indicates the grain size distribution to be strongly coarse-skewed. This is somewhat misleading in that the skewness is relative to the mean grain size which is much finer than the other sediment samples.

TABLE 4. PARTICLE SIZE DISTRIBUTION (%) FOR SEDIMENT CORES TAKEN INSIDE AND OUTSIDE OF BOAT TRACK, 11-23-80.

Core	Depth (cm)	(mm) ϕ	1.000 0	.500 1	.250 2	.125 3	.063 4	<.063 5
Out-1	0-2		0.49	1.22	14.66	62.52	4.65	16.44
"	2-5		0.70	0.78	12.07	65.19	3.88	17.36
"	5-10		0.50	1.37	14.69	67.44	3.27	12.73
"	10-15		0.56	2.21	17.62	63.95	4.20	11.46
Out-2	0-2		0.70	2.07	20.75	59.60	3.24	13.63
"	2-5		0.41	1.10	14.17	61.27	5.21	17.83
"	5-10		0.60	1.54	21.44	61.69	3.18	11.55
"	10-15		0.23	0.82	12.81	64.92	4.74	16.48
In-1	0-2		0.11	1.65	20.71	62.78	2.87	11.89
"	2-5		0.15	1.72	34.04	55.58	2.01	6.50
"	5-10		1.06	2.58	22.76	57.91	4.26	11.43
"	10-15		3.68	5.16	24.96	46.86	2.49	16.84
In-2	0-2		0.21	0.95	18.77	72.96	2.86	4.25
"	2-5		0.07	1.56	29.02	61.03	2.42	5.91
"	5-10		0.10	1.63	20.51	60.45	3.41	13.90
"	10-15		0.57	3.05	26.25	58.16	2.39	9.57
"	18-23		1.59	1.50	6.04	37.04	4.49	49.34

TABLE 5. STATISTICAL PARAMETERS OF GRAIN SIZE FOR SEDIMENT CORES TAKEN INSIDE AND OUTSIDE OF BOAT TRACK, 11-23-80.

Core	Depth (cm)	Mean (M_z)	Median (Md)	Sorting (σI)	Skewness (SK_1)
Out-1	0-2	2.8	2.6	0.92	+0.31
"	2-5	2.9	2.6	0.90	+0.31
"	5-10	2.5	2.5	0.67	+0.11
"	10-15	2.4	2.4	0.73	+0.02
Out-2	0-2	2.5	2.4	0.80	+0.17
"	2.5	2.9	2.6	0.92	+0.29
"	5-10	2.4	2.4	0.74	+0.12
"	10-15	2.9	2.6	0.88	+0.36
In-1	0-2	2.4	2.4	0.73	+0.11
"	2-5	2.2	2.2	0.73	+0.17
"	5-10	2.4	2.4	0.80	+0.04
"	10-15	2.6	2.4	1.29	+0.13
In-2	0-2	2.4	2.4	0.57	+0.11
"	2-5	2.3	2.3	0.69	+0.09
"	5-10	2.6	2.4	0.83	+0.21
"	10-15	2.3	2.3	0.78	+0.06
"	18-23	3.4	3.7	0.94	-0.48

Comparisons between the sediments in the boat track and those in the adjacent, undisturbed bed suggest an increase in the fine fraction (<5 phi) in the top 5 cm in the bed. Both mean and median statistics as well as the skewness measure also indicate a slight decrease in grain size in the surface layers. The particle size distribution as well as the statistical parameters indicate little apparent change with depth for sediments inside the boat track. Considering the large maount of bioturbation observed throughout the summer months, this homogeneity is not unexpected. Zieman (1976) indicates a slight decrease in fine material (4 phi) in a single boat track and a considerable decrease in fines in one continually kept open from repeated scouring by small boats. He did not indicate however, the depth to which his samples were taken. Most probably the slight differences observed in our study area between the vegetated and unvegetated zones are the result of insufficient wave and current scouring actions, baffled in part, by the existing vegetation adajcent to the denuded cut. Whatever the actual differences however, they do not appear after seven months to be sufficient to inhibit the revegetation by the submerged grasses.

CONCLUSIONS

Patterns of revegetation of the boat track observed in this study indicate that in a mixed assemblage of *Zostera marina* and *Ruppia maritima* it is *R. maritima* that is the more rapid colonizer. Revegetation by *R. maritima* and *Z. marina* occurred primarily as lateral growth from the unimpacted vegetation at the sides of the cut although any vegetation, either *Z. marina* or *R. maritima*, which is not completely uprooted by the boat propeller may serve as a focal point for new growth. *Zostera* seedlings were obsserved in the fall throughout the boat track and their presence indicates a potentially important mechanism for revegetation.

Analysis of sediment data indicate that the sediments both inside and outside of the boat track dominated by fine sands and are fairly homogeneous to depths of approximately 20 cm. Active bioturbation of the sediments appears a likely mechanism for this homogeneity. The urooting of the vegetation by the boat propeller therefore initially had little net effect on the grain size of the sediments. After seven months however, there was some evidence that in the top 5 cm there were finer particles outside the boat track than inside.

Extractable sediment pore water nutrient concentrations suggest comparable levels of inorganic phosphorus both inside and outside of the cut with little observable change with depth. Nitrate and nitrite levels were highest in the top 2 cm of sediment, due possibly ot oxidation of ammonium, with no significant difference between the vegetated and unvegetated areas. Ammonium levels were, conversely, lowest in the top 2 cm and appeared to have accumulated to higher levels below 5 cm depths inside the boat track when compared to outside. It would not appear from these data that differences in sediment nutrients were limiting the vegetative regrowth into the cut.

The more rapid regrowth observed in this study for *Ruppia maritima* as compared to *Zostera marina* parallels that observed by Jones (1968) and

Phillips (1960) for *Halodule wrightii* as compared to *Thalassia testudinum*. Seven months after the disturbance, however, the *R. maritima* had spread over less than half of the 1 m wide denuded zone. Since *R. maritima* experiences little net growth during the winter months at this latitude, it would appear that at least two growing seasons may be required for recolonization by *R. maritima*. Recolonization by *Z. marina* appears to take significantly longer. Certainly little regrowth was evident during the study period. This suggests that three years or more are required for revegetation, with a part of the regrowth a result of recruitment by seedlings and relic turions not originally removed from the sediment. These time intervals appear comparable to those suggested by Zieman (1976) for *H. wrightii* and *T. testudinum*.

Considering the patterns of revegetation observed in this study, in mixed areas of *Zostera marina* and *Ruppia maritima* succession after a physical disturbance proceeds from a *R. maritima* community to a *R. maritima*-*Z. marina* community. It is not uncommon in many areas to observe homogeneous stands of *R. maritima* in otherwise mixed zones of submerged vegetation. Possibly these patches of vegetation are sites of previous physical distrubances from boat propellers, ray activity, etc. that have been initially recolonized by *R. maritima*.

Patterns of revegetation may vary from site to site and season to season depending on a number of factors. Because of *Ruppia maritima*'s less extensive rhizome mat as compared to *Zostera marina*, souring by wave action during severe storm events may selectively uproot the *R. maritima* leaving largely *Z. marina*. At other times both species may be removed. The period when a disturbance occurs also can impact the intitial revegetation successional stages. Disturbances during the fall may result in little regrowth for the next six months. If severe storm activity occurs during the winter months, erosion of these areas unprotected by the rhizome mats may preclude revegetation for quite some time. In extreme conditions heavy boating activity combined with highly exposed conditions may result in the permanent loss of vegetation.

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CHAPTER 6

GROWTH OF ZOSTERA MARINA L. SEEDLINGS UNDER LABORATORY CONDITIONS OF INCREASED NUTRIENT ENRICHMENT

by

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ABSTRACT

The effect of increased nutrient on growth of Zostera marina seedlings was laboratory tested by adding two different concentrations of a slow release fertilizer, Osmocote. Three different application rates were used with the two formulations (18:6:12 and 14:14:14) of Osmocote by hand placing these amounts into peat pots holding one seedling.

The addition of fertilizer to the substrate markedly stimulated the growth of seedlings in the laboratory. Fertilization promoted growth both in the increased leaf length and in vegetative production of increased number of shoots but did not result in an increase in the leaves/shoot. The nitrogen rich formulation (18:6:12) produced less growth than the equal balance formulation (14:14:14). For both formulations, the highest concentrations exhibited greater growth than the other concentrations of the same formulation. Results of this experiment corroborated results from previous work suggesting that addition of nutrients in the sediment can stimulate growth and seagrasses are nutrient limited in some types of sediments.

INTRODUCTION

Laboratory culture of Zostera marina is essential for some types of experimental studies of life-history, physiology, growth, and reproduction and potentially has value for production of plants to reestablish grass beds in denuded areas. In both cases, it is desirable to know how fertilization affects growth under controlled conditions. Fertilization of marsh plants and seagrass under field conditions is known to stimulate growth (Raymont, 1947; Buljan, 1957; Valiela, 1975; Valiela et al., 1973, 1976; Valiela and Teal, 1974; Garbisch et al., 1975; Orth, 1977; Orth and Moore, 1982) but effects under laboratory conditions have not been studied previously.

The objective of the experiment described here was to evaluate the effect of two fertilizers at several concentrations on growth of seedlings under laboratory conditions. The experiment was preliminary in nature since no information on laboratory culture of seedlings was available on which to base a refined experimental protocol.

MATERIALS AND METHODS

Seedlings for this experiment were collected 11 March 1980 from a grass bed at Guinea Marsh, York River, Va. Seedlings were manually uprooted by divers and collected in plastic bags.

Soil for the experiments was collected from the same site as the seedlings and placed in 5 x 5 cm square peat pots supported in plastic greenhouse trays. A sediment core was removed from selected pots. The core in a Gelman filter centrifuge tube (0.45 μm glass fiber filter) was centrifuged for 10 minutes. The filtrate was analyzed for NH_3^+, NO_2^-, NO_3^- and PO_4^- with a Technicon Autoanalyzer II (Kopp and McKee, 1979). The core sample represented 21% of the total sediment and pore water in the peat pot. Seedlings were planted in the peat pots and held in flowing estuarine water for two weeks. Seven groups of 52 seedlings were then selected on 20 March 1980 for the experiment.

The fertilizers selected for the experiment were two formulations of Osmocote®, one with a N:P:K ratio of 18:6:12, the other 14:14:14. Osmocote was selected because it is a slow release fertilizer. No attempt was made to determine whether there was a slow release of the fertilizer under the water logged conditions of the experiment. It was assumed that all nitrogen and phosphorus were released in a form available to the plants. Each fertilizer was applied at three application rates (g/m^2) (Table 1). Application rates for each formulation of Osmocote were calculated to provide the same three amounts of total nitrogen; 12.5, 25, and 50 g/m^2. The appropriate amount of

TABLE 1. SUMMARY OF FERTILIZER APPLICATION RATES. SEDIMENT NITROGEN AND PHOSPHORUS CONCENTRATIONS WERE CALCULATED FROM APPLICATION RATE AND CONCENTRATION IN FERTILIZER ASSUMING TOTAL AVAILABILITY OF BOTH NUTRIENTS

Treatment	Fertilizer	Application Rate (g/m^2)	 (g/peat pot)	Nitrogen g/m^2	Phosphorus g/m^2
A	None	0	0	0	0
B	14:14:14	89.3	0.23	12.5	12.5
C	14:14:14	178.6	0.46	25	25
D	14:14:14	357.1	0.91	50	50
E	18:6:12	69.4	0.18	12.5	4.2
F	18:6:12	138.9	0.35	25	8.3
G	18:6:12	277.8	0.71	50	16.7

fertilizer was placed on the sediment surface of each peat pot and tamped into the substrate while the pot was in the air. Pots were immediately returned to the holding tank receiving flowing water. Another group of plants which received no fertilizer served as the control. Crude dividers (fiberglass) were placed in the holding tank to segregate all treatments. The holding tank was located in a greenhouse and received about 50% incident light at the water surface.

Ambient estuarine water was pumped from the York River, Va. and filtered to 10 μm with GAF filter bags. Flow rate was adjusted to insure several volume turnovers per day. Despite filtration the water in the holding tank was turbid because fine particles predominated in the incoming water. Actual light intensity at the sediment surface of the peat pots was not measured, but was presumed equal for all treatments. Any shading effects of the holding tank were not controlled.

The day following fertilization the number of leaf blades/plant and length of longest blade were determined and recorded. At two week intervals thereafter, the plants were wiped gently with fingers to remove detritus and epiphytes. Number of shoots, leaf blades/shoot, and length of longest blade on oldest shoot were determined. The seventh and final measurement was made on 13 June 1980.

Leaf blade lengths for each treatment were compared for each measurement interval by one-way analysis of variance and Duncan's multiple range test. Number of leaf blades/plant and number of shoots were analyzed by nonparametric methods. All statistical analyses were performed using SAS packaged programs on the William and Mary IBM computer system.

RESULTS

During the acclimation period and the first growth interval, the temperature averaged 10.3° and 10.8°C respectively while salinity declined from 17.8 to 15.7 ‰ (Table 2). Mean temperature increased in each succeeding growth period to 27.3°C during the final interval. Salinity declined to 14.9 ‰ during the third growth interval, and then increased to 17.9 ‰ during the final period. Throughout the study, dissolved oxygen measured by Winkler titration during the midday period usually exceeded saturation. The most extreme value was 27.4 mg/l observed on 7 June. Observed oxygen concentrations exceeded saturation in 92% of the observations over the entire study period. Supersaturation is believed to have resulted from the photosynthetic activity of the *Zostera* plants plus that of the diatoms and other microphytes growing within the system. The extreme values of dissolved oxygen during the final growth period resulted largely from the microphytes since *Zostera* growth was reduced.

The measured concentrations of each inorganic nitrogen form and total phosphorus, in micromoles, are presented in Table 3 for prefertilization samples and samples collected at the end of the 12-week growth period. Ammonia was the principal form of nitrogen present both before and after fertilization whereas nitrite was present in extremely small amounts. In all

TABLE 2. MEAN TEMPERATURE, SALINITY AND DISSOLVED OXYGEN CONCENTRATIONS DURING ACCLIMATION AND GROWTH PERIODS FOR *ZOSTERA* GROWTH/FERTILIZATION STUDY.

	T (°C) mean SD	S (°/oo) mean SD	D.O. (mg/l) mean SD	over-saturation frequency	percent
acclimation period	10.3 ± 2.6	17.82 ± 0.56	11.50 ± 1.13	5/6	83
1 III - 4 IV	10.8 ± 2.1	15.67 ± 0.69	12.40 ± 2.29	10.13	77
5 III - 18 IV	14.3 ± 1.3	15.83 ± 0.76	13.09 ± 3.21	12/14	86
19 IV - 2 V	19.6 ± 2.0	14.86 ± 0.28	14.10 ± 2.92	14/14	100
3 V - 16 V	22.3 ± 3.5	15.41 ± 1.24	13.01 ± 5.26	13/14	93
17 V - 30 V	23.8 ± 1.3	15.97 ± 0.77	12.00 ± 3.43	12/12	100
3 V - 13 VI	27.3 ± 1.5	17.91 ± 1.03	18.93 ± 3.95	11/11	100
				77/84	92

TABLE 3. NUTRIENT CONCENTRATIONS (μm) IN SEDIMENT PORE WATER BEFORE AND AFTER THE GROWTH PERIOD.

Treatment		NH_4^+	NO_3^-	NO_2^-	PO_4^{-3}
3/24/80 Prefertilization		204 ± 46	1.97 ± 0.59	0.83 ± 0.49	41.1 ± 27.4
6/17/80 Post Growth					
Control	A	10300 ± 6590	1290 ± 924	1.60 ± 0.88	0.74 ± 1.28
14:14:14	B	4460 ± 1150	366 ± 203	1.30 ± 1.77	14.6 ± 8.1
14:14:14	C	4990 ± 1130	493 ± 208	0.20 ± 0.18	42.5 ± 31.1
14:14:14	D	5675 ± 3180	594 ± 198	1.05 ± 0.25	42.9 ± 30.4
18:6:12	E	7450 ± 5240	1100 ± 1560	1.44 ± 0.36	5.7 ± 4.7
18:6:12	F	5220 ± 1150	1430 ± 2090	1.14 ± 1.07	16.3 ± 10.8
18:6:12	G	4400 ± 950	245 ± 64	0.62 ± 0.48	27.1 ± 8.5

treatments including the control, ammonia-N and nitrate-N concentrations in the sediment were greatly elevated above those observed prior to fertilization. Sedimentary phosphorus concentrations after the growth period were below those observed prior to fertilization in all but two cases (Treatments C and D). The standard deviations for all samples were large.

At the start of the experiment, each seedling consisted of a single shoot with an average of 4.1 leaves/shoot (maximum of six leaves/shoot). The number of shoots/plant increased slowly during the experiment until, at the end of the study, the mean numbers of shoots/plant were 1.2 in the control and from 2.8 to 3.5 in the fertilized groups (Fig. 1). After four weeks, a few control plants developed three shoots but at no time did more than 12% of the control plants have three shoots, while 65% of the control plants still had only one shoot. By the end of the growth period, 84% had only a single shoot (Table 4).

In all experimental groups, the number of plants with three shoots increased throughout the experimental period. After 6 to 8 weeks, some plants would develop four or more shoots per plant (Table 4). At the end of the 12 week study period, 30-47% of the plants fertilized with 14:14:14 and 21-28% of the plants fertilized with 18:6:12 had four or more shoots (Table 5). The tendency for production of multiple shoots was clearly enhanced when plants were fertilized, and especially so when 14:14:14 Osmocoat was applied.

The maximum number of leaves/shoot observed during the experiment was eight, but usually shoots had four to six leaves. At the start of the experiment the mean number of leaves/shoot was 3.8-4.3. The mean number of leaves/shoot was not obviously different at the end of the study (3.7-4.4). No attempt was made to monitor sloughing of leaves.

The average length of the longest leaf (hereafter referred to as average leaf length) was 8.6 to 9.2 cm at the start of the experiment and increased throughout the study period. The average leaf lengths were not significantly different among the treatments until after 4 weeks growth (Table 6) but, thereafter, three to four groups of treatments were definable by a Duncan's multiple range test. After 8 weeks, all experimental treatments were significantly different from the control group and assorted into two groups: treatments B, C, D, and G exhibiting greater average leaf length than treatments E and F. The latter difference was much smaller than the difference from the control group.

The growth increment for each time interval was calculated as the average leaf length at time (t+1) minus the average leaf length at time (Table 7). The initial growth increment was small, increased to a maximum in interval 2 and 3, and then declined. During the final interval, growth had almost ceased in those treatments receiving the most fertilizer, and was very low in controls and all other treatments. Greatest overall growth increments occurred in the treatments receiving the highest amounts of fertilizer.

The mean leaf length for each treatment was plotted against nitrogen applied at the start of the experiment (Fig. 2A). The treatments receiving

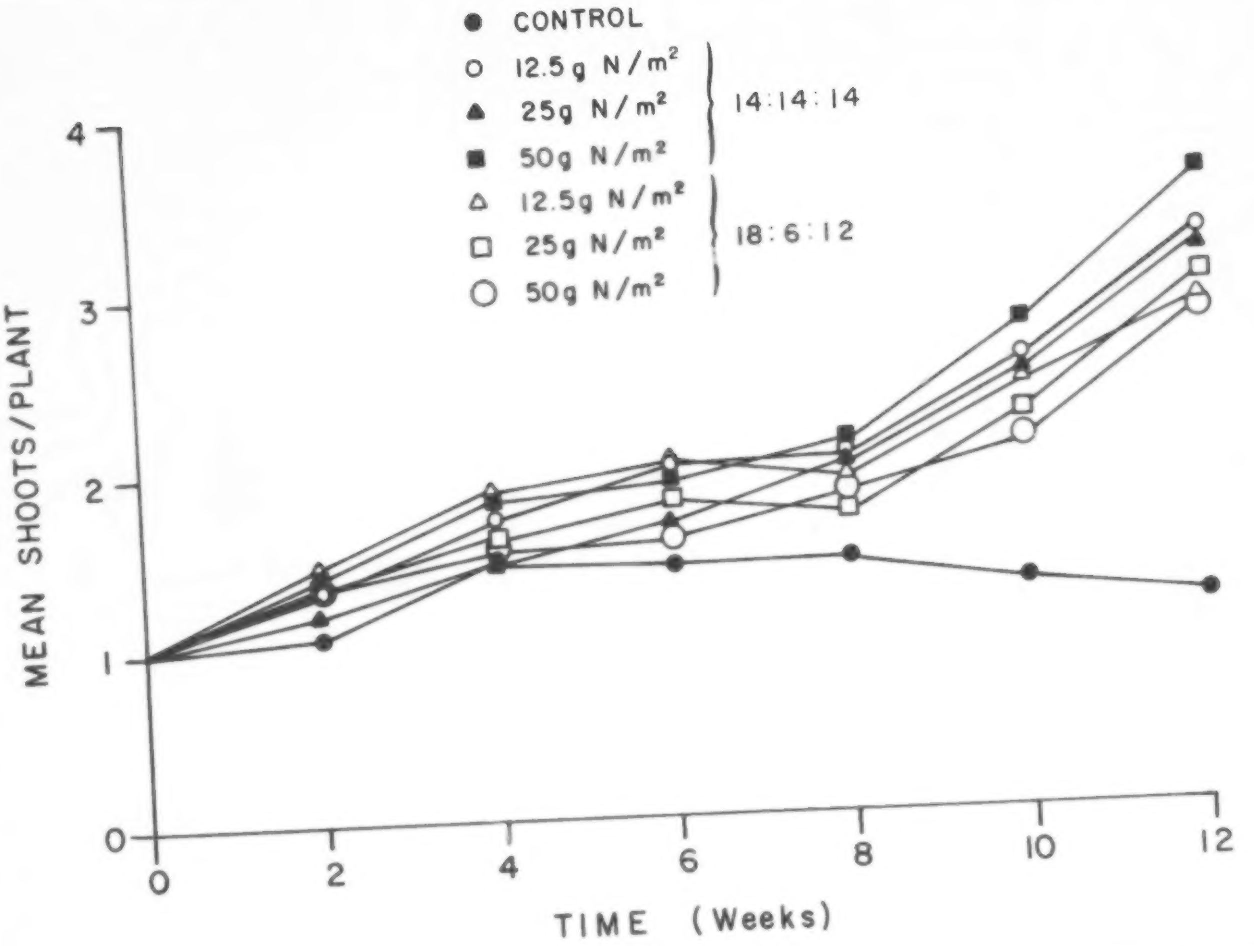

Fig. 1. Mean number of shoots/plant observed during the course of the experiment for each of the six treatments and control.

TABLE 4. NUMBER OF SHOOTS/PLANT AT THE END OF EACH GROWTH PERIOD.

Growth Period (weeks)	Treat.	Number of Shoots								Number of Plants	Mean Number of Shoots/Plant
		1	2	3	4	5	6	7	8		
2	A	43	7							50	1.1
4		32	13	4						49	1.4
6		34	9	6						49	1.4
8		33	14	2						49	1.4
10		38	9	2						49	1.3
12		41	6	2						49	1.2
2	B	35	16	1						52	1.4
4		22	21	8						51	1.7
6		16	20	15						51	2.0
8		14	23	11	2					50	2.0
10		5	22	15	7	1				50	2.5
12		4	5	26	9	4	1		1	50	3.2
2	C	40	9							49	1.2
4		30	14	3						47	1.4
6		21	19	4	1					45	1.7
8		14	18	11	2					45	2.0
10		8	16	15	4	2				45	2.5
12		4	9	16	11	2	3			45	3.2
2	D	35	11	4						50	1.4
4		18	19	10						47	1.8
6		16	20	10	1					47	1.9
8		15	17	12	2	1				47	2.1
10		9	11	15	6	4	1			46	2.7
12		5	5	15	10	6	6			47	3.5
2	E	27	14	3						44	1.5
4		14	22	8						44	1.9
6		15	18	6	4					43	2.0
8		16	18	6	3					43	1.9
10		5	20	12	4	2				43	2.5
12		4	13	16	7	2	1			43	2.8
2	F	29	17							46	1.4
4		25	14	7						46	1.6
6		20	14	9	1					44	1.8
8		20	16	6	1					43	1.7
10		12	16	10	3	2				43	2.2
12		5	13	13	5	6	1			43	2.9
2	G	33	16	1						50	1.4
4		29	14	7						50	1.6
6		24	18	5						48	1.6
8		21	16	8	3					48	1.9
10		15	20	8	3	1	1			48	2.1
12		6	11	21	9				1	48	2.8

TABLE 5. PERCENTAGES OF PLANTS WITH EACH OBSERVED SHOOTS/PLANT AFTER THE 12 WEEK GROWING PERIOD.

Treatment		Number of Shoots/plant			
		1	2	3	4+
Control	A	84	12	4	0
14:14:14	B	8	10	52	30
	C	9	20	36	36
	D	11	11	32	47
18:6:12	E	9	30	37	23
	F	12	30	30	28
	G	13	23	44	21

TABLE 6. COMPARISONS OF AVERAGE LEAF LENGTH FOR EACH TREATMENT AT EACH TIME INTERVAL. VALUES UNDERLINED WERE NOT SIGNIFICANTLY DIFFERENT BASED ON DUNCAN'S MULTIPLE RANGE TEST.

TIME							
0 wks	G 9.2	B 9.2	C 9.1	D 9.1	A 8.8	F 8.7	E 8.6
2 wks	E 11.4	C 11.2	D 11.0	G 11.0	B 10.9	A 10.8	F 10.7
4 wks	E 17.7	G 17.6	D 17.3	C 17.1	B 16.0	F 15.7	A 14.9
6 wks	G 26.4	C 24.3	D 23.9	E 22.9	B 21.7	F 21.3	A 17.1
8 wks	G 29.0	D 28.8	C 28.0	B 26.5	E 25.1	F 24.8	A 18.0
10 wks	D 33.2	G 32.7	C 31.1	B 30.6	F 28.7	E 28.2	A 20.3
12 wks	D 33.4	G 32.8	B 31.9	C 31.8	E 29.1	F 29.0	A 21.5

TABLE 7. BI-WEEKLY GROWTH INCREMENTS (cm) IN AVERAGE LEAF LENGTH DURING EACH GROWTH INTERVAL.

Treatment		Growth period (wks)						Overall
		2	4	6	8	10	12	Total
Control		2.0	4.1	2.2	0.9	2.3	1.2	12.7
14:14:14	12.5 gN/m^2	1.7	5.1	5.7	4.8	4.1	1.3	22.7
	25	2.1	5.9	7.2	3.7	3.1	0.7	22.7
	50	1.9	6.3	6.6	4.9	4.4	0.2	24.3
18:6:12	12.5	2.8	6.3	5.2	2.2	3.1	0.9	20.5
	25	2.0	5.0	5.6	3.5	3.7	0.3	20.3
	50	1.8	6.6	8.8	2.6	3.7	0.1	23.6

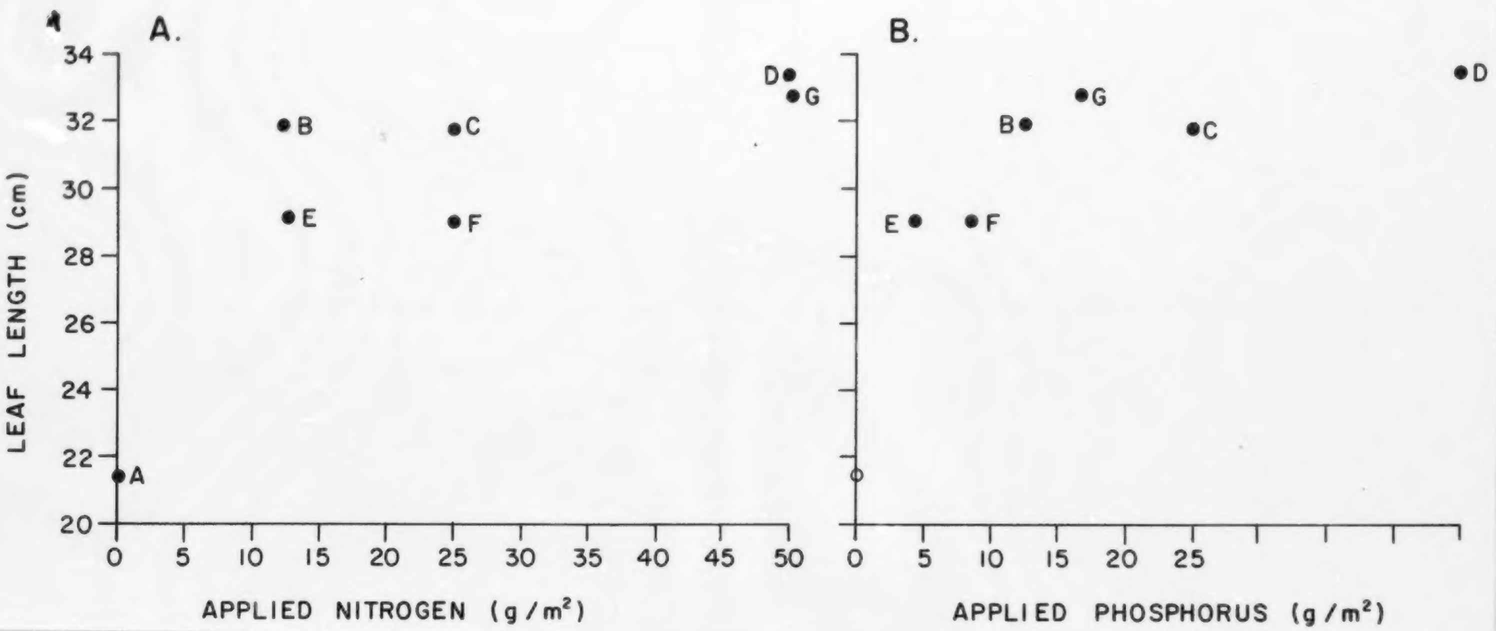

Fig. 2. Mean leaf length (cm) for each treatment plotted against applied nitrogen (g/m^2) (A) and applied phosphorus (g/m^2) (B) at the start of the experiment.

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14:14:14 Osmocoat (treatments B, C, and D) exhibited better growth than did those receiving 18:6:12 Osmocoat (treatments E, F, and G) except at the highest application rate. The mean leaf length for each treatment was also plotted against the amount of phosphorus applied at the start of the experiment (Fig. 2B). Leaf length increased with increasing application rate of phosphorus up to 16.7 g/m^2. Clearly, at equal application rates of nitrogen, less growth occurred in the treatments receiving less phosphorus. Increased applications of nitrogen had little effect on leaf length.

DISCUSSION

In his discussion of the seasonal pattern of the life cycle of *Zostera marina*, Setchell (1929) identified five seasonal segments for growth and reproduction. These segments are 1) a cold rigor period at temperatures below 10°C, 2) a vegetative period from 10-15°C, 3) a reproductive period from 15-20°C, 4) a heat rigor period at temperatures above 20°C, and 5) a recrudescent rigor period as temperatures decline below 20°C. The present growth study spanned temperatures from 10°C to 27°C, thus covering the first four seasonal segments. During the first growth period when temperatures hovered around 10°C, growth occurred at a slow rate. Maximal growth occurred during the next two periods when temperatures increased to about 20°C, corresponding to Setchell's seasonal segments 2 and 3. Sexual reproduction was not observed, but was not expected since seedlings do not reproduce sexually. Vegetative reproduction (production of new shoots) was observed during all periods of the experiment, but was especially pronounced during the first 4 weeks (temperature 10.8 to 14.3°C) and the final 4 weeks (temperature 23.8 to 27.3°C) (Fig. 1). As temperatures exceeded 20°C, leaf growth continued as well as vegetative shoot addition, but at a slower rate, and as the temperature increased over 25°C, growth nearly ceased.

There was no trend in mean number of leaves/shoot or maximum number/shoot at any time during the study. New leaves were continuously appearing on each shoot, but after five or six appeared, the rate of new leaf addition was about equal to the loss of old (outer) leaf blades so that the leaves/shoot remained constant. Total leaves/plant increased simply because the number of shoots increased over the study period from around four at the start to an average of about 12 after 12 weeks, though plants with seven shoots might have 28-30 leaves.

Several conclusions can be drawn from this study. Obviously, addition of fertilizer to the substrate markedly stimulated growth of seedlings in the laboratory. This agrees with observations of enhanced growth of *Zostera* in natural beds fertilized with commercial fertilizers (Orth, 1977). More recently, Orth and Moore (1982) have shown that fertilization enhances survival and growth of transplanted *Zostera* plugs. Fertilization promotes growth both in the sense of increased leaf length and in vegetative production of increased number of shoots, but does not lead to an increase in leaves/shoot. Orth and Moore (1982) also reported a striking increase in number of shoots in fertilized transplants of *Zostera*.

With respect to increased leaf length, the nitrogen-rich phosphorus-poor formulation (18:6:12) produced less growth than the equal balance formulation (14:14:14). For both formulations, the highest concentrations produced greater growth than the other concentrations of the same formulation. Only the 50 g/m^2 application rate of 18:6:12 formulation yielded growth in leaf length equal to that observed in plants receiving the 14:14:14 formulation.

The production of multiple shoots/plants was pronounced in all fertilized groups. Only 4% of the control plants exhibited three shoots/plant whereas more than 60% of all fertilized plants exhibited three or more shoots/plant; indeed more than 20% exhibited four or more shoots/plant. With the 14:14:14 formulation 30 to 47% of the plants had four or more shoots/plant, the proportion increasing with increasing application rate. For the nitrogen-rich formulation, 21 to 28% of the plants possessed four or more shoots/plant, but there was no clear relationship to application rate.

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